Training report

Engineer degree in Agronomy AgroSup Dijon

Effect of the pod production dynamic of the cocoa tree and inoculum sources on the Frosty Pod Rot (*Moniliophthora roreri*) development

(Internship undertaken from 01/31 to 07/30/13)

Veromanitra RAKOTOBE
2nd year

Jean-Philippe GUILLEMIN: tutor, AgroSup Dijon
Jacques AVELINO: internship supervisor, Cirad
Philippe TIXIER: internship supervisor, Cirad
Mariela LEANDRO-MUÑOZ: internship joint supervisor, Catie

2013

Abstract

Frosty Pod Rot (FPR) is caused by the fungus *Moniliophtora roreri*. This is a serious disease and can cause up to 80% of yield loss. Currently, the disease management control is tough: it consists in weekly removal pods and diseased pods must be buried. Until now, very few studies were conducted on the factors influencing pods infection, but it is known that climatic factors such as temperature, precipitation relative humidity and moisture play a role in this infection (Leandro-Muñoz 2011). This paper aims to study the effect of the pod production dynamic of the cocoa tree and inoculum sources on the FPR development in relation with the climatic factors. Three clones were studied: CATIE R4, CC137 and Pound 7 with differences of resistance to the disease. The results confirmed that CATIE R4 is the most resistant and Pound 7 the most sensitive. Bagging the diseased pods, which was equivalent to the elimination of the local inoculum on the plot, showed a significant difference. This result also confirmed previous results and supports the current disease control. But the local inoculum must be studied at a bigger scale and not only at the plot one. Our results suggested studying it at the plot scale and nearby plots scale. Our results also showed that the generation is crucial in the response to the disease: according to the generation, a clone can behave like another one, meaning be more resistant or sensitive. This point is of very importance and underlines the interest of studying cocoa phenology to find clones able to produce in climatic periods unfavourable to the fungus. Then, in order to understand how the climate influences changes of status of pods (from healthy to diseased), we constructed a binomial Generalized Linear Model (GLM) highlighting climatic key periods of the change of status (relatively to the date of labelling studied pods, the date and the duration of the climatic variable considered). We demonstrated that, according to the clone, key periods are completely different. To better understand these periods, we constructed a more complete GLM based on the key periods identified previously. The results did not lead to satisfactory conclusions because they did not correspond to the results obtained on the field; moreover, we did not include the variable of precipitation neither the distinction between pods into bags and pods without bags in this model. Despite the absence of solid conclusions on the effect of climate on FPR development, we underlined the importance of some climatic variables and their complex relation. Variables of precipitation and moisture must be included in the following models. Other analysis must be run according to the generation and inoculations in controlled conditions should be realized in order to understand which variable is the most influent. This should permit to build other models to highlight maybe non-linear relations between variables.

Key words: cocoa, *Moniliophtora roreri*, climate, local inoculum, phenology

Thanks

I would like to thank Jacques AVELINO and Philippe TIXIER, my internship supervisors for their intelligence, availability and assistance to each stage of my internship. This assistance was more than beneficial. I really appreciated their kindness and good mood throughout my short presence in the Catie.

I want to thank Mariela LEANDRO-MUÑOZ too, for her support in the end of my internship. Carrying out this internship enabled me to realize how I an interested in investigation. I hope that I have a chance to work with people like them in my career.

I must also thank the other trainees and students with whom I had unforgettable moments. This placement year was wonderful because I have been given the chance to meet wonderful people, work from day to day with them and live in a peaceful and natural environment.

Table of contents

List of figures

List of tables

List of annexes

Introduction

Generalizations about cocoa

Theobroma cacao L. originated in the headwaters of the Amazon River (eastern Ecuador and Peru) (Pound 1938; Lanaud *et al*. 2003; Bartley 2005). Archeological records indicate that it was domesticated at least 2,600 years ago in Mesoamerica (Motamayor *et al*. 2002; Bartley 2005). *T. cacao* is member of the large family of *Malvaceae*, which is comprised of the former families *Sterculiaceae* (cocoa and kola), *Bombacaeae* (baobab, durian and kapok), *Malvaceae sensu lato* (cotton, hibiscus and okra), and *Tiliaceae* (basswood) (Judd *et al*. 2002). It is one of the 22 species of the genus *Theobroma*. The species is divided into 10 genetic groups: Amelonado, Contamana, Creole, Curaray, Guiana, Iquitos, Marañón National Nanay and Purús (Motamayor *et al*. 2008).

T. cacao L. is an evergreen tree with an average height of 5 to 8 meters and dense foliage. Its fruits are called pods and are harvested year-round. Its productive life is from 25 to 30 years. Cocoa is considered as an agroforestry crop, since it is usually grown under shade trees forming systems based on two or more perennial crops. The importance of shade trees in the perennial crop plantations lies in the fact that these trees offer products and incomes as fruits or timber when the cocoa prices are low. Besides, shade trees contribute in the maintenance of the biodiversity in the systems (Beer *et al*. 1998).

The culture of cocoa in the world

The 2010/2011 cocoa season has experienced an estimated record surplus of 343,000 tonnes following a notable deficit of 132,000 tonnes the year before. Global production is estimated to have increased substantially, by nearly 19%, compared with the previous season, reaching over 4.3 million tonnes, while demand for beans also reached a record level of over 3.9 million tonnes (ICCO 2012).

On the production side, global record output of 4.3 million tonnes is attributed mainly to a bumper harvest in Africa which rose by nearly 740,000 tonnes to 3.2 million tonnes with the main contributors being Côte d'Ivoire and Ghana, the world's two largest cocoa producing countries. Africa has not failed to reinforce its status as the premier cocoa-producing region, accounting for nearly 75% of world output but contrasting with regional trends of other cocoa growing regions. Production increased by 43,000 tonnes in the Tropical America to 559,000 tonnes (representing 13% of world output) but declined by 109,000 tonnes in the Asia and Oceania region, to 524,000 tonnes (or 12% of global production) (ICCO 2012).

Cocoa production limits

Its production spreads over two periods of the year: July to February and March to June. Currently, Costa Rica's production has been decreasing for few years even though it is maintaining around 500 tonnes a year. The reason for this drop is mainly due to plantation abandon because of moniliasis (*Moniliophtora roreri*) which causes Frosty Pod Rot (FPR). The current lack of technical assistance is limiting the capacity to face this disease.

Moniliophtora roreri: a fungus causing Frosty Pod Rot disease

M. roreri is considered as a hemibiotrophic fungus, due to its cycle that goes through two stages: a biotrophic phase, from the germination of the spores to the intercellular invasion of the pod, and a necrotic phase causing growth reduction of the pods and ending with the fungus invasion to the cells causing the appearance of internal and external necrosis (Thevenin and Trocmé 1996). *M. roreri* is able to thrive under a wide range of environmental conditions, from sea level to over 1,000 meters above sea level and from very dry to very humid zones (Evan 1981). Spores are the only infective propagules of *M. roreri*, and the fruits of *Theobroma* and *Herrania* species are the only susceptible organs (Evans 1981a). *T. cacao* L. fruits are infected when they are young (0 to 3 months old), and become less susceptible as they mature. Fruit maturity occurs 5 to 6 months after pollination. External fruit symptoms may include small water-soaked lesions, deformation, premature ripening, and chocolate-colored spots. Fruits that are infected in very early stages usually die. In advanced infections, the internal pod tissues appear to form a compact mass surrounded by a watery substance as a result of tissue maceration, which causes the total loss of the seeds. Chocolate-coloured spots develop a layer of white mycelium within 4 to 5 days, which becomes darker as the spores mature. After about 3 months, these fruits become dry and mummified on the trees and remain attached to the trunk. These fruits become the major source of inoculum for consecutive infection waves that then occur over a long period of time. Spores are produced in great abundance on diseased fruits (over 7 billion per fruit), and become widely distributed after they are released. They are multifunctional, serving for genetic exchange, but also for dispersal and infection, as well as for survival. Wind is the main mode by which spores disperse. However, wind dispersal fails to explain the spread of FPR over significant distances and geographical barriers. This spread is easier to explain by human activities. The long period of fruit colonization prior to the manifestation of visible symptoms allows apparently healthy, systematically infected fruits to be selected and transported for use as a source of planting material (Evans 1986).

Its center of origin is located in Colombia, and from there the pathogen spread to 12 countries in tropical America: Ecuador (formerly considered as the place of origin), Venezuela, Panama, Costa Rica, Nicaragua, Peru, Honduras, El Salvador, Guatemala, Belice, Mexico and recently Bolivia (Phillips-Mora *et al*. 2006).

When the disease appeared in Peru in 1987, it rapidly became the most important disease problem, displacing witches' broom disease (caused by *M. perniciosa*) (Evans *et al*. 1998). The impact of FPR from Panama to Mexico has been substantial, and has invariably become the main yield-limiting factor for cocoa production in affected countries, with frequent reports of pod losses higher than 80%. But for the moment, the disease is still limited to Spanish-speaking countries, where farmers know it from the characteristic external symptoms (Evans 2007).

Monilia disease control

FPR has been reported to be twice as destructive as black pod (*Phytophthora palmivora*) (Desrosiers and Diaz 1957), and more dangerous (Orellana 1954) and difficult to control (Aranzazu 2000) than witches' broom (*M. perniciosa*). Schematic models have been presented for FPR to highlight weaknesses in its life cycle, particularly where low-cost, cultural control can be employed most effectively (Evans 1973; Evans 1981a; Evans 1981b). A model of field dynamics of *M. roreri* was developed later, which also underlines the need for frequent harvesting to reduce inoculum levels (Leach *et al*. 2002). However, with added "real world" variables, the control equation becomes much more complex and, ultimately, is dependent on the socio-economic status of the farmer. Thus, whereas some measures (cultural, chemical and biological) have been very effective on an experimental scale (Barros 1980; Porras *et al*. 1990; Krauss and Soberanis 2001), only those based on cultural practices (periodical removal of diseased pods, pruning of the cocoa and shade trees, maintenance of the drainage system, etc.) are being adopted by smallholders.

In Costa Rica, weekly removal improved yields by more than a factor five in comparison with no removal (Porras *et al*. 1990) and in Colombia, weekly removal proved to be both most efficient and economical in comparison with monthly and twice-weekly removal (Cubillos and Aranzazu 1979). In Peru, weekly removal of diseased pods (moniliasis, witches' broom and black pod) reduced the incidence of diseases significantly in comparison with fortnightly removal. In three fields, moniliasis was decreased by 26 to 41% and the cumulative effect was a consistent yield increase from an average of 504 to 660 kg/ha/yr. Returns compensated for increased labour costs. Weekly pod removal was 32% more profitable (Soberanis *et al*. 1999).

Cultural management is currently considered to be the main practical means of control for smallholders (Soberanis *et al*. 1999). The frequency with which infected pods can be removed from the field is essential for an effective control of the disease (Cubillos and Aranzazu 1979; Porras *et al*. 1990; Soberanis *et al*. 1999). In fact, the frequency and cost of these practices (especially weekly removal of diseased pods) have played a major role in discouraging their use, especially when cocoa prices are low. Nevertheless, regular removal of diseased pods has a disadvantage: all current cocoa plantations are hybrids, meaning that it is necessary to multiply them by culture *in vitro* or vegetative multiplication, which are not necessarily mastered.

The devastating effects of FPR on cocoa have been dramatic and are well documented across different countries, including Colombia in 1817 (Anonymous 1832), Ecuador in 1918 (Rorer 1918), Costa Rica in 1978 (Enriquez *et al*. 1982), and Mexico in 2005 (Philips-Mora *et al*. 2006). Current losses are highly variable, ranging from 10 to 100% (Anonymous 2006), and depend on factors such as length of time the disease is present in a site, age of plantation, crop and disease management, presence of neighbouring affected plantations and weather conditions. In a global context, the current annual loss from FPR is small, but the potential danger presented by the disease is enormous (Phillips-Mora and Wilkinson 2007).

Monilia previous studies: very few information about the fungus development

Currently, little is known about biology of the fungus and disease development. Besides, FPR is restricted to South America countries, so that international investigation is little implicated in its management. A lot of behaviours and favourable factors of the fungus remain unknown.

But it is known that phenologic and climatic factors play a role in the fungus development. Temperature is the most influential factor on the growth rate of the fungus (Moore-Landecker 1996). According to Leandro-Muñoz (2011) when the fruit is young and temperature high, the period of infection is shorter than when the fruit is more mature. Its increase causes the raise of chemical and enzymatic activity that accelerates vitamins synthesis, amino acids and other metabolites; however, excessive heat may inactivate these activities and stop the growth. Concerning *M. roreri*, the ideal range for growth and sporulation of colonies in culture medium V8 is 24 to 28°C (Herrera 1988). According to Hawker (1950), the optimum temperature range for fungi sporulation is always lower than the optimum temperature range for growth. Leandro-Muñoz (2011) concluded that air temperature and pod temperature from the early days of infection were the only microclimatic variables related with growth rates of the disease in different generations of pods.

As well as temperature, very little is known about the other climatic factors (precipitation, relative humidity, wind, pH, light, free water) that favour *M. roreri* development.

Objectives of the study

This study is part of a thesis investigation conducted in Costa Rica, on 3 clones (CATIE R4, CC137 and Pound 7) with different levels of resistance. The trial began on May 2012 and was conducted until the end of July 2013. We aimed to study microclimatic factors effects (temperature, relative humidity, humidity and precipitation) related to pod production dynamic (generations of pods) and local inoculum quantity on the fungus development and impact on cocoa.

In this paper, we explain how microclimate conditions affect FPR development and how local inoculum is transmitted from a generation of pods to another one. Our results showcase climatic influence on infection appearance of the disease, and then climatic favourable conditions for its development and transmission. We also study cocoa phenology, which could have an influence on the disease development.

Cocoa phenology study

The 6 months previous results showed that moniliasis is a monocyclic disease ie with only one infection stage and meaning that only the initial inoculum is affecting disease evolution. J-form disease development curves obtained suggested also only one infection stage, followed by symptoms expression brought forward in time. Thus, epidemic disease progression should be allowed by successive generations of pods, each generation transmitting spores to another one. This hypothesis is confirmed with the removal of pods effect: when diseased pods are removed, initial inoculum disappears for following generations and hence, this has a huge

effect. Thus we highlight the interest to study cocoa phenology. It should permit us to feature potential correlations between close generations, even if climate does not necessarily play a role.

Local inoculum study

Climatic factors study would also permit us to showcase the range and set of favourable conditions to the fungus development, and highlight which conditions are favourable to epidemic infection and then to symptoms appearance. But behaviour of inoculum sources is not well known. Removing diseased pods is currently the most effective control but we aimed to quantify and characterize its effect on next generations.

Material and methods

Studied area

The trial was conducted at the experimental farm La Lola, located in 28 Millas, district of Batan, canton of Matina, province of Limon, on the Atlantic centre Coast of Costa Rica. La Lola belongs to the Tropical Agricultural Research and Higher Education Centre (CATIE). The site is located 40 m.a.s.l, 10°06' latitude North and 83°23' longitude West. Average rainfall (1949-2010) is 3575 mm with a decrease on March and September. Temperature ranges (1952-2010) between 20.5°C and 30.0°C. Relative humidity is above 91% on average in the same period. The prevailing climate is of Caribbean influence: it is humid with a dry marked season, quite cloudy with few sunny hours and water excess soils according to the period of the year. The warmest months are May and June, whereas December and January are the coldest. According to Holdrigde (1967), the zone belongs to the humid and cloudy tropical forest. Theses conditions are very favourable to moniliasis development, so that the site is under high inoculum pressure with observed incidences around 80% of diseased pods in commercial plots with no control.

Trial

The experiment L6 (42 clones) was planted in La Lola by CATIE's Cocoa Breeding Program with the purpose of selecting clones with high productivity, resistance and other attributes. Its total area is 1.5 hectares. In our experiment, only 3 clones with various levels of resistance to monilia were studied: Pound 7, CC137 and CATIE R4 (in order of increasing resistance). Each clone is replicated 4 times. The experimental unit comprises 8 cocoa trees. Planting distance is 3 x 3 m. The shade is distributed unevenly and is composed of trees of Erythrina (*Erythrina* sp.) and guaba (*Inga edulis*) (Figure 1).

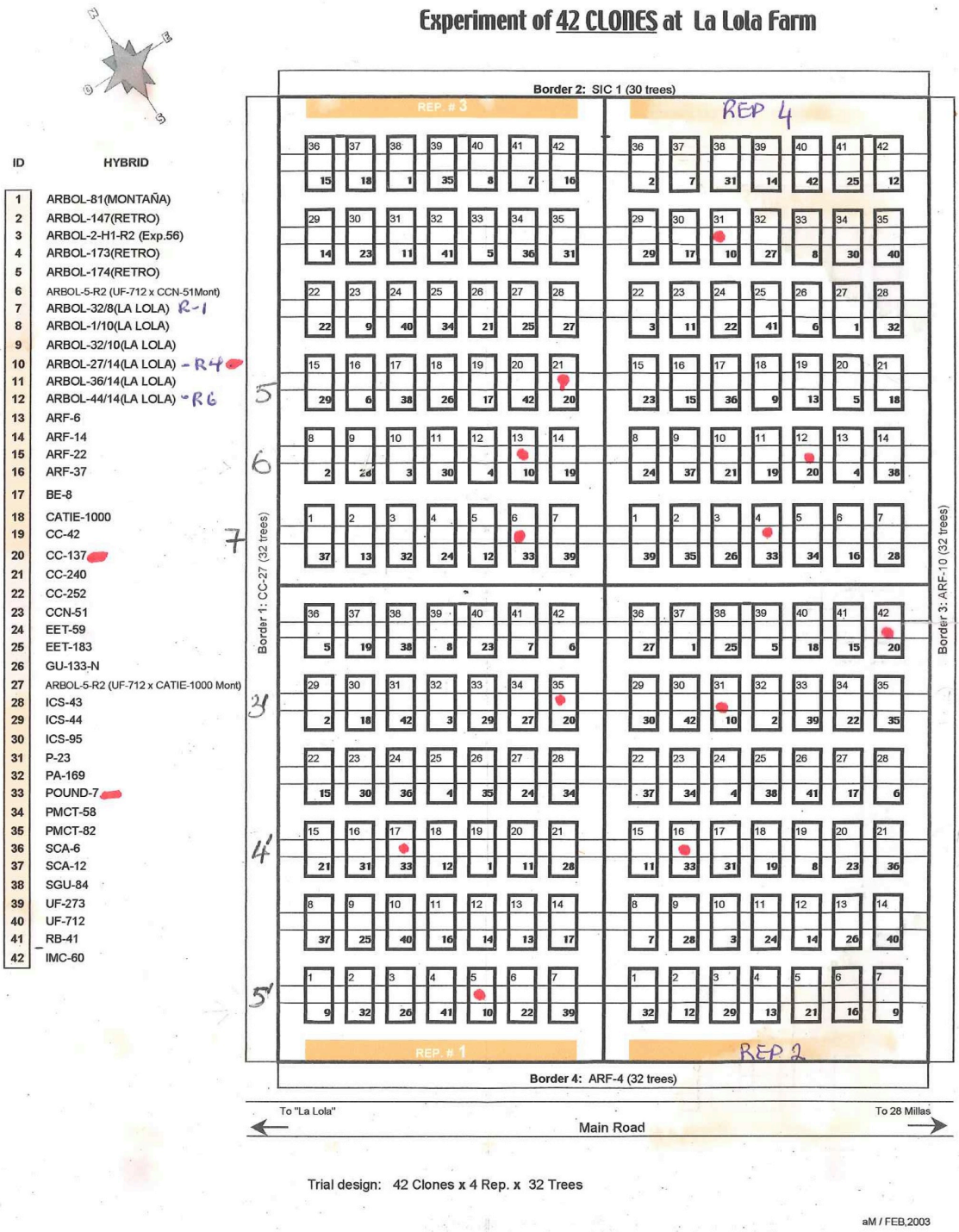


Figure 1. Plan of the experiment of 42 clones at La Lola Farm

Basic principle

We weekly followed each pod evolution for each clone, from its birth (labelling on the tree at about 2 months of age) to its removal or harvest (5-6 months). For some pods (2 repetitions out of 4), the local inoculum has been eliminated (diseased pods were put into bags to prevent moniliasis spores from dispersing). Each week, we reported the labelled pods state (healthy, moniliasis symptoms, spores, other disease, cherelle wilt – a physiological disease quite common but not contagious, removed or harvested), thus obtaining the pods evolution all over

their life. At the same time, microclimatic data (temperature, relative humidity and humidity) were recorded every 15 minutes with a Hobo climatic station.

Studied factors

Clones

Three clones among the 42 with different levels of resistance were observed: Pound 7 is the most sensitive to moniliasis. Its production is good but its potential of production has fallen down severely because of the disease, practically reaching zero. Its annual production is around 700 kg/ha/year with 86% loss because of the disease.

CC137 recorded yield losses lower than 30% since years, but since FPR development, it recorded a considerable yield reduction with an original annual production of 1400 kg/ha/year and a decrease of 43%.

CATIE R4 has been the variety presenting the best output and the best resistance to moniliasis during years of evaluation by the CATIE's Cocoa Breeding Program. Its annual production is around 2000 kg/ha/year with a yield loss of 12% caused by moniliasis.

Generations

Every week, nascent pods between 3 and 8 cm (1 to 2 months of age) were labelled since the first generation (29 May 2012) until the 55th (12 June 2013). The pods were observed every week until they were removed because of moniliasis, healthy harvested, affected by other disease (mainly *Phytophtora*) or cherelle wilt. The moniliasis symptoms are registered in Figure 2.

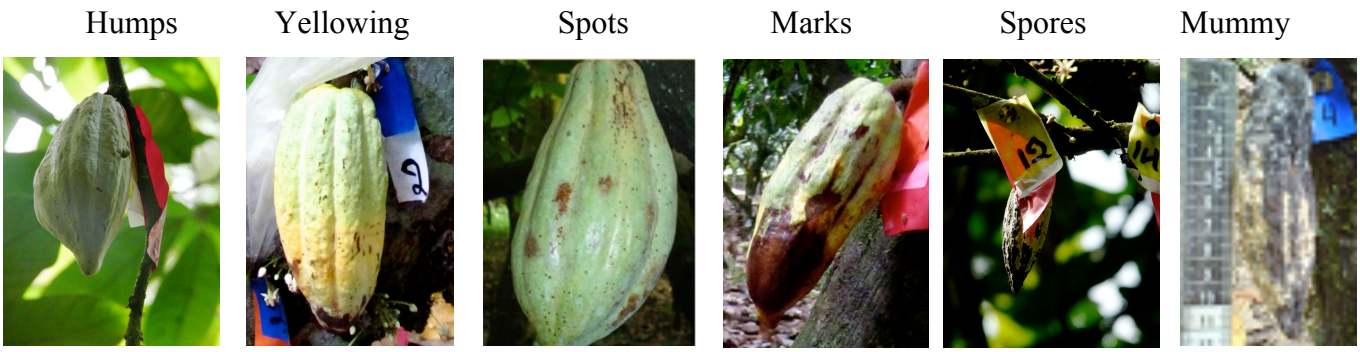


Figure 2. External symptoms of moniliasis

Bagging

We put into bags pods presenting monilia symptoms or spores to eliminate local inoculum and reduce spores dispersion on some plots, so that we could assess the bag effect on the disease development (Figure 3).

Figure 3. Plot with bag treatment (Pound 7 repetition 4)

Height

During labelling the pods, we registered their height on the tree (low ie < 1.5 m or high ie > 1.5 m) in order to see if it could have an effect on the disease dispersion.

These 4 studied factors were observed on 2 repetitions.

Pods observation

From the labelling of the pods, we weekly observed each pod until they are 10 weeks old. From the 11th week (4 to 5 months of age), they were evaluated only one week out of two because the older they are the more they become resistant to moniliasis (Leandro-Muñoz 2011).

Microclimatic data

Microclimatic data were registered with a HOBO station. The station was set up in the repetition 4 of a CC137 plot. Three temperature, 4 humidity and 2 relative humidity and temperature sensors were installed in the middle of the 8 trees of the plot (Table 1 and Figure 4). It was programmed to record every 30 seconds the climatic data and make an average of these data every 15 minutes.

Table 1. Sensors identification

Position	Sensor	Serial number	Height (m)
1	Temperature & relative humidity	9835243	1.5
2	Temperature &	9835244	1.5

	relative humidity		
3	Temperature	1228065	2
5	Temperature	1228066	2
6	Temperature	1228029	1
7	Humidity	1217670	1.25
8	Humidity	1217680	1.25
9	Humidity	1217679	1.25
10	Humidity	1217682	1.25

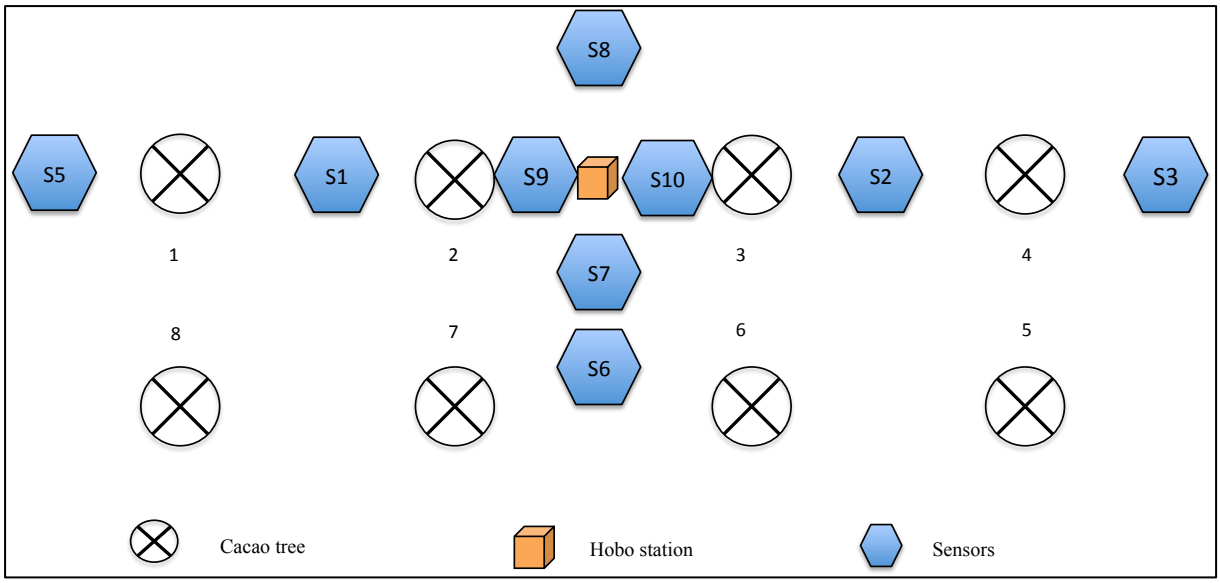


Figure 4. Diagram of the sensors in plot CC137 repetition 4

Pods observation and climatic data input

Two databases were developed: one for the monilia symptoms observations and the other one for the climatic data.

The monilia symptoms database is identical to the observation sheet. For each pod, we registered the initial date (corresponding to the date of labelling), its height and then every date of observation. We also registered every date of change of state until its removal or harvest.

The climatic database is equal to the microclimatic data we recorded with the climatic station. We also calculated averages and amplitudes of temperature, relative humidity and humidity. Unfortunately, the sensors were defective at some times, so that we made correlations with another climatic station situated in la Lola. Calibration curves are available in Annexe 1.

Studied variable

We studied the probability of change of status of each pod (from healthy to diseased) at different periods (0-30, 30-60 and 60-90 days after labelling).

For that, climatic variables were used: relative humidity average, amplitude of temperature and temperature average.

Statistical analyses

Statistical analyses were operated with R 2.10.1 GUI. We realised a generalized linear model (GLM) to:

- Calculate the probability of change of status of each pod, which obeys a binomial law (0 = healthy; 1 = diseased). We ran this analyse on each pod (9,500)
- Analyse the factors effects (Clones, Generations, Bagging, Height) on the change of status at removal date of all pods
- Identify climatic variables (which variable, from what date and for how long) best associated with the change of status at different periods (0-30, 30-60, and 60-90 days after labelling)
- Try to built a complete model with best significant climatic variables

Results

Pods observation on the field permitted to cumulate more than 10,000 data in a year, representing 55 generations of fruits. However, the following results were calculated on 48 generations, ie about 9,500 pods.

Factors effects on change of status of pods at removal date

First analyses permitted to establish the probability of change of status of pods. It is the probability of infection of pods by *M. roreri*, considering different factors: Generation, Clone, Height, Treatment (bagging) and their interactions. Results show that this probability is explained by every factor and the interactions Generation: Height, Generation: Clone, Generation: Treatment, Height: Clone and, to a lesser extent, by Height: Treatment. The interaction Clone: Treatment is not significant (Table 2).

Table 2. Level of significance of the factors and their interactions in change of status of pods

Factors and interactions	Level of significance	AIC variation
Generation	2.2e-16***	0
Height	2.2e-16***	0
Clone	2.2e-16***	0
Treatment	2.2e-16***	0
Generation: Height	2.317e-10***	40
Generation: Clone	6.832e-16***	59
Generation: Treatment	2.255e-06***	0
Height: Clone	0.0001335***	13
Height: Treatment	0.03786*	2
Clone: Treatment	0.3347	2

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Theses results highlight that every factor has a significant effect on the change of status of pods. Everything is significant because there are thousands of pods, but according to AIC variations, interactions have the most significant effects. So that the more relevant factors affecting change of status are the interactions.

The interactions Generation: Height, Generation: Clone and Generation: Treatment are significant. It highlights that height, clone and bagging effects depend on the generation because we observed differences between height, clone and the treatment according to whether the generation appeared under favourable conditions to the disease or not. The existence of favourable conditions to the disease development was tested in a following analyse.

Generations don't perform the same because they are not under same climatic conditions given that they were not born at the same period. In order to illustrate behaviour difference between our clones, we realized infection curves for each clone for a generation (interaction Generation: Clone). The next figures underline that some clones can reach the resistance of another clone, according to the generation, thus illustrating the escaping phenomenon to the disease. So the very importance of the generation is underlined (Figure 5 to Figure 8).

Figure 5 represents how behave the three clones for generation 6. The graph shows that every clone, comparing with the average of the 5 previous years, has a low infection rate. So according to the generation, every clone is able to resist to the disease.

Figure 6 shows that CC137 behaves like CATIE R4, the more resistant to monilia, with a maximal average infection rate of 20% whereas it reached until 43% in average in the previous years.

Figure 7 represents generation 19. The clone CATIE R4 behaves like CC137, meaning with a high infection rate, reaching almost 60%.

Figure 8 represents generation 24 and the clone CC137 behaves as susceptible to the disease as Pound 7, reaching an infection rate of 80%.

These figures underline that CC137 is the most susceptible to behave like CATIE R4 or Pound 7 according to the generation. This behaviour cannot be genetic because it would be observed more times. But these variations of behaviour are favoured by climatic conditions. So we can see that the generation is very important in the behaviour of the clone face to the disease. According to the generation, differences of behaviour are observed and it could be a mean to escape from the disease. Some times are favourable to disease development with a high pressure of monilia and some other times are not favourable. This fact highlights the importance of focusing on cocoa phenology to select clones able to produce in periods of non-infection.

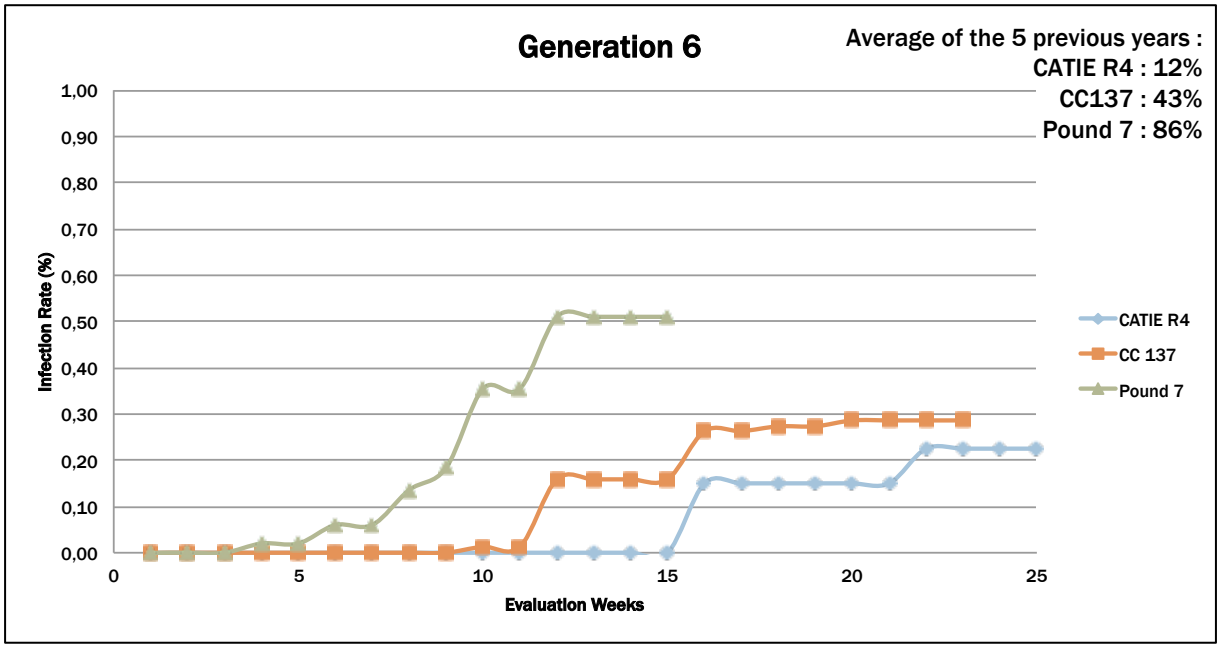


Figure 5. Interaction Generation: Clone for generation 6

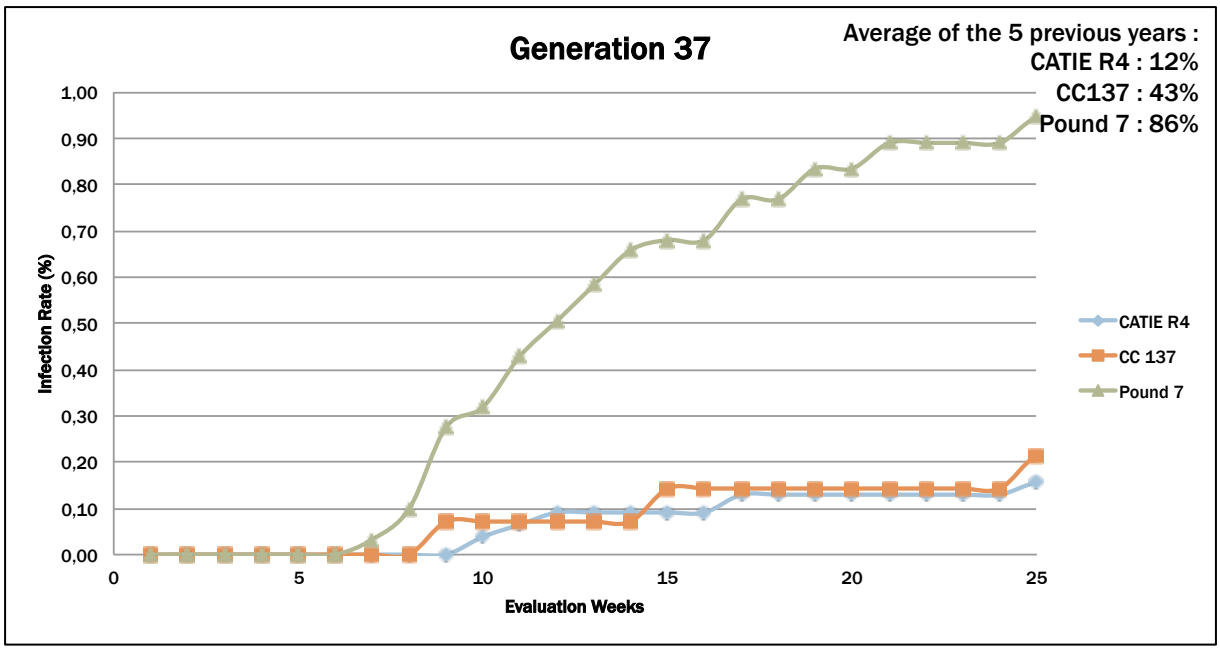


Figure 6. Interaction Generation: Clone for generation 37

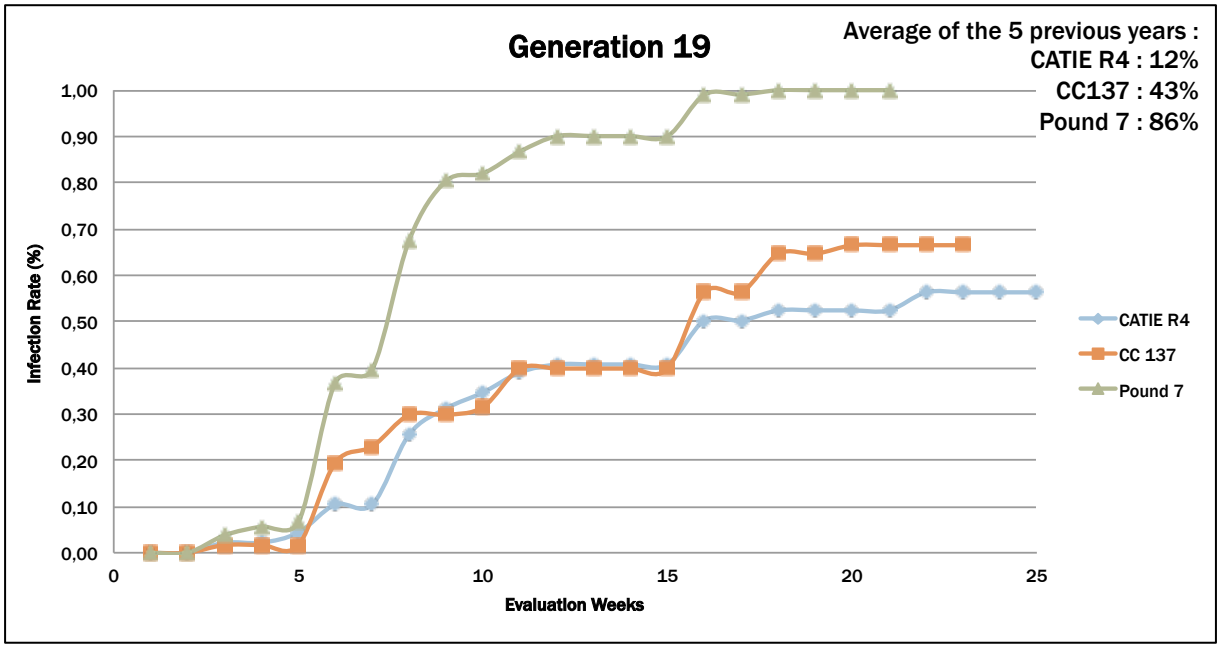


Figure 7. Interaction Generation: Clone for generation 19

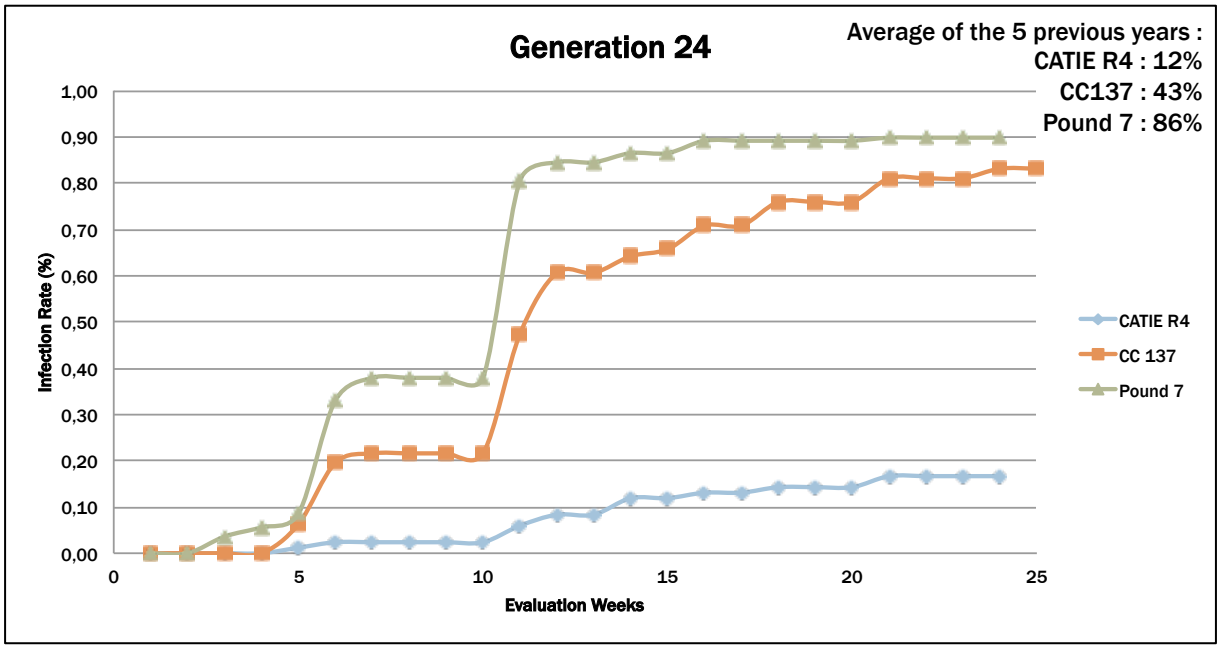


Figure 8. Interaction Generation: Clone for generation 24

Pod position in the tree is significant too. However, this criterion does not permit to establish a disease management strategy but only gives an indication of infection risk of the pods according to their position. We didn't test the way of its effect, but we already know that it depends on the dynamic of dispersion of the spores. According to Leandro-Muñoz (2011), spores tend to mass in the lower part of the tree. Two ideas are hypothesized:

- Spores tend to fly up during the day but if relative humidity increases, spores fall down
- Precipitations involve spores at the bottom of the tree

Theses two hypotheses lead us to think that pods located in the lower part of the tree are more affected by monilia than fruits in the upper parts.

The three clones behave also differently face to the disease. It confirms previous results according to which our clones present great differences in resistance to the disease (Leandro-Muñoz 2011).

Bagging pods has also a significant effect on disease development. Eliminating the local inoculum thus has an effect on disease development. It confirms the interest of regular removal.

Infection rate of each clone by moniliasis

Infection rate curves for each clone (with or without bags and per generation) were realized (Figure 9 to Figure 20). Each graph only represents 24 generations at once because generations have a 6-months life expectancy. So that younger generations are not present at the same time than first generations.

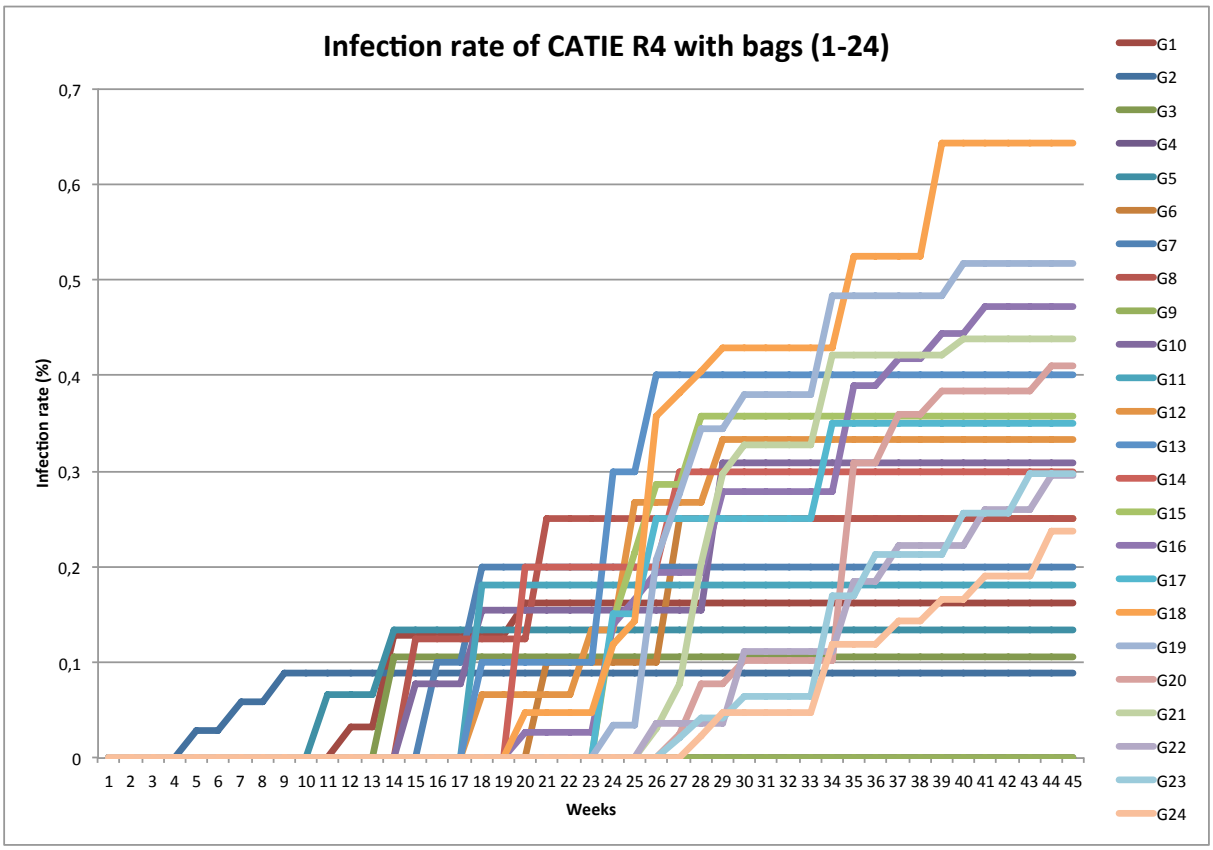


Figure 9. Infection rate of CATIE R4 with bags from generation 1 to 24

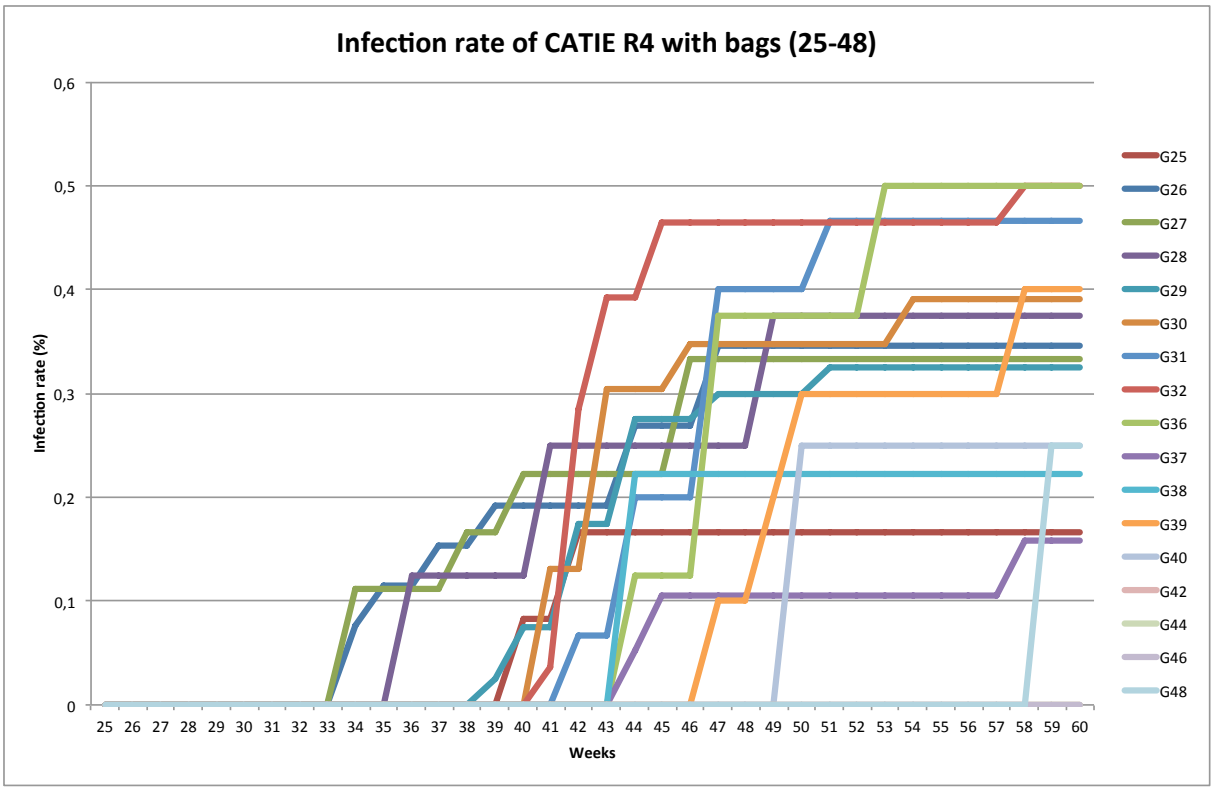


Figure 10. Infection rate of CATIE R4 with bags from generation 25 to 48

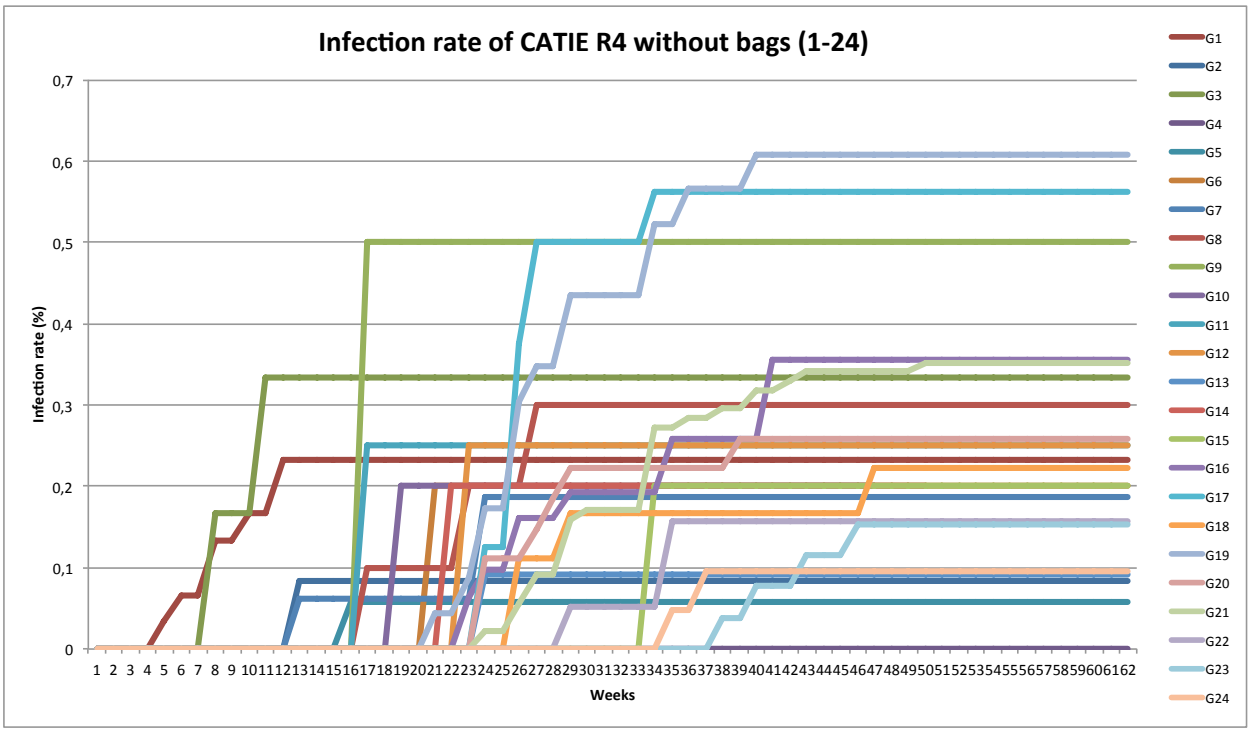


Figure 11. Infection rate of CATIE R4 without bags from generation 1 to 24

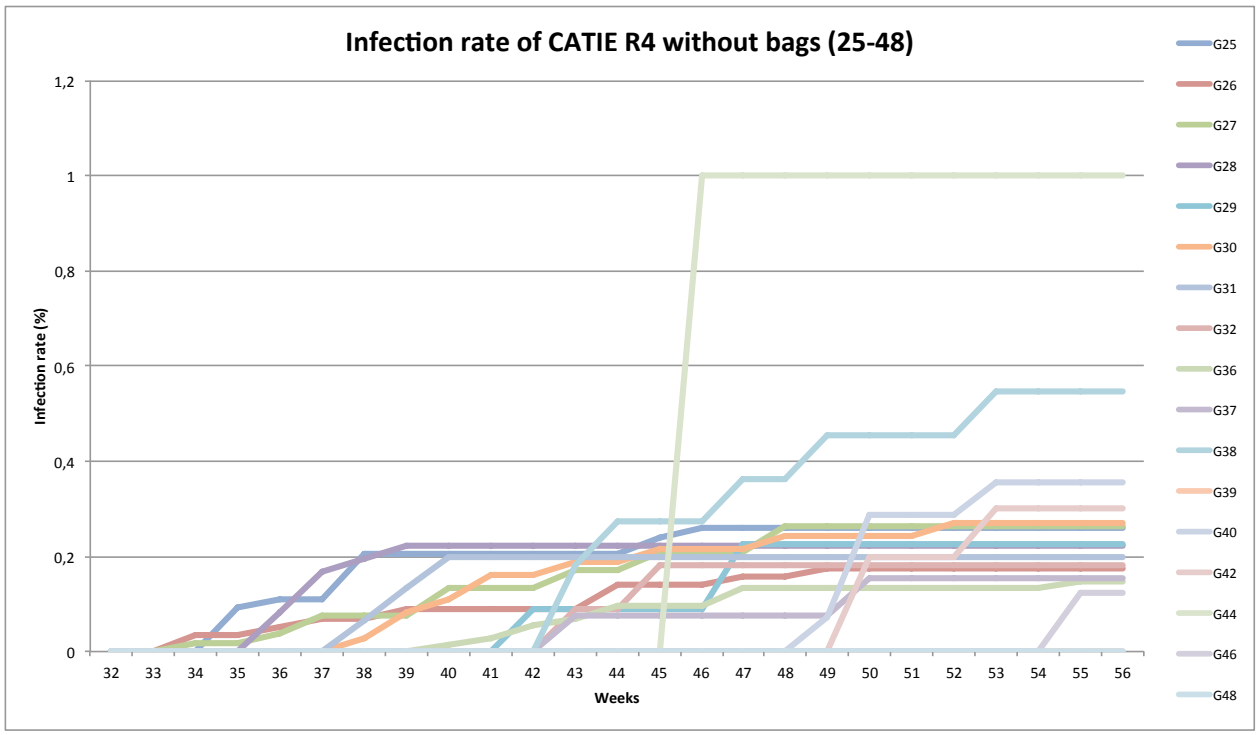


Figure 12. Infection rate of CATIE R4 without bags from generation 25 to 48

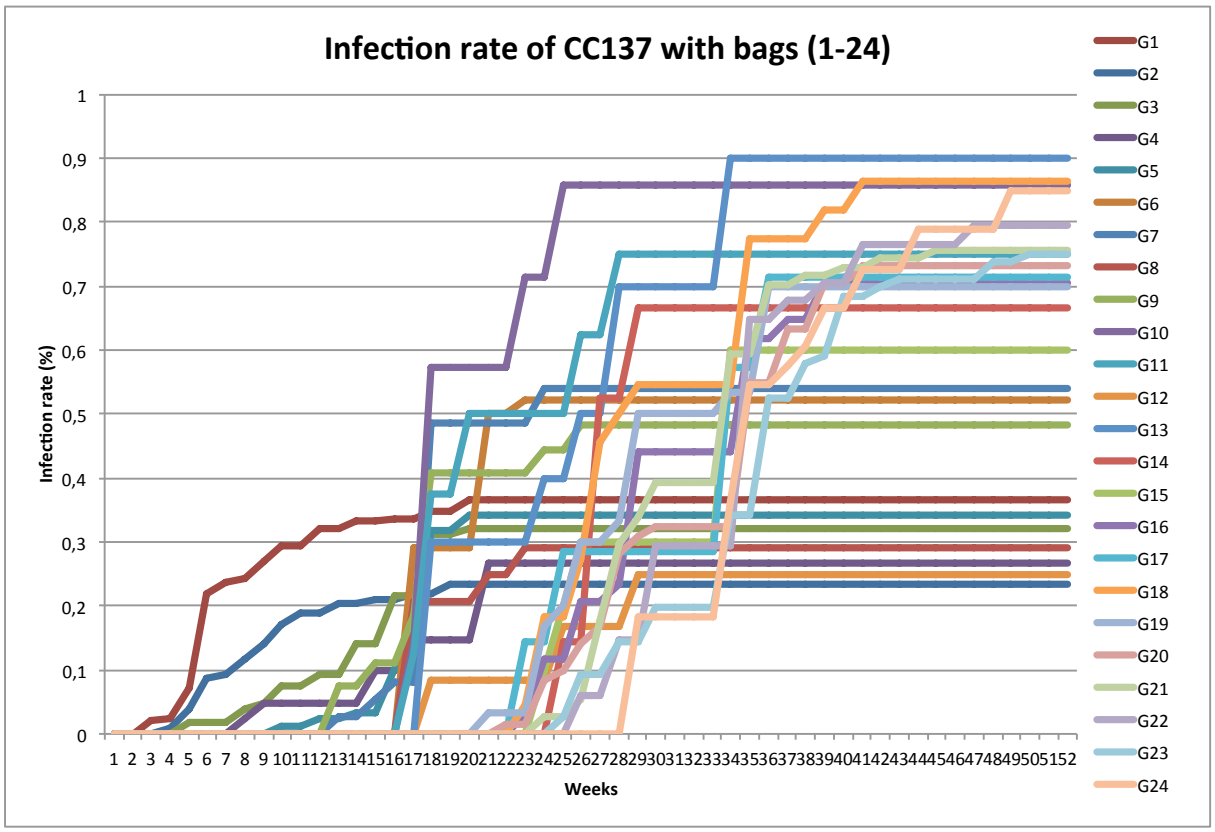


Figure 13. Infection rate of CC137 with bags from generation 1 to 24

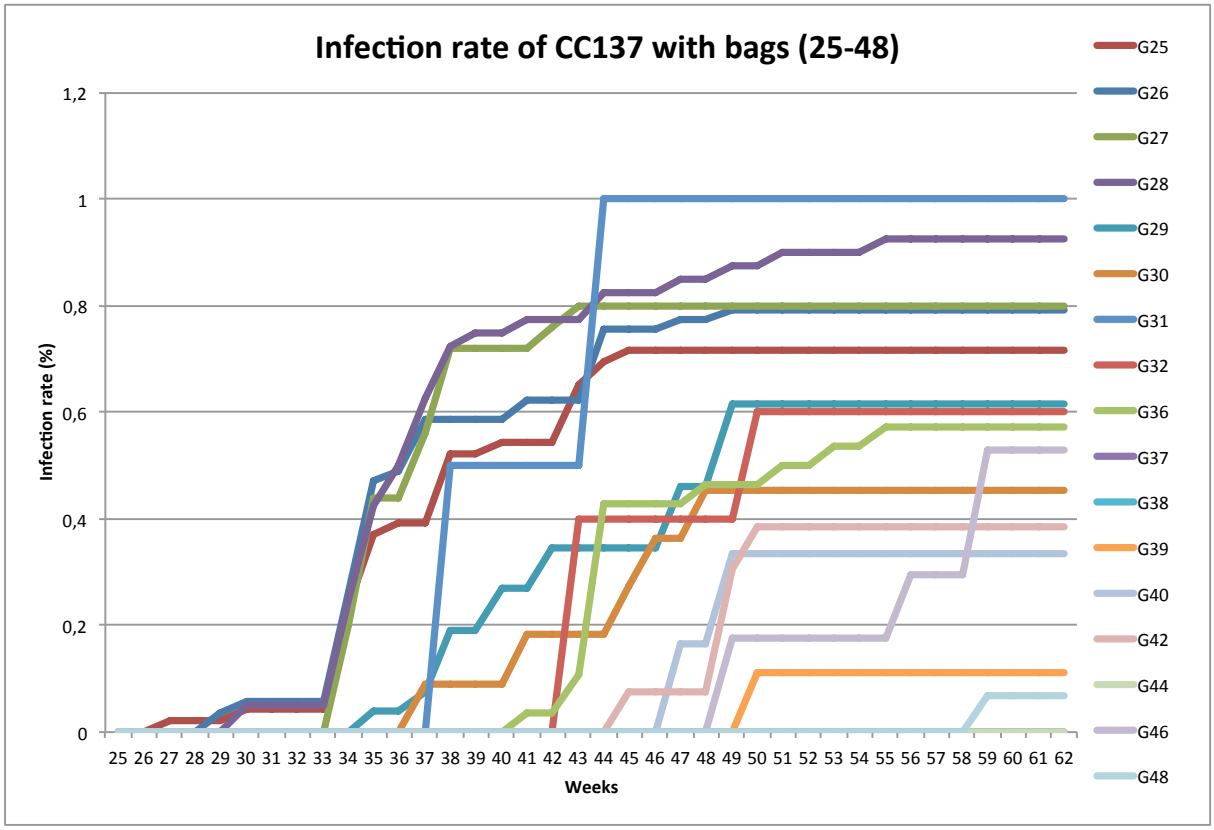


Figure 14. Infection rate of CC137 with bags from generation 25 to 48

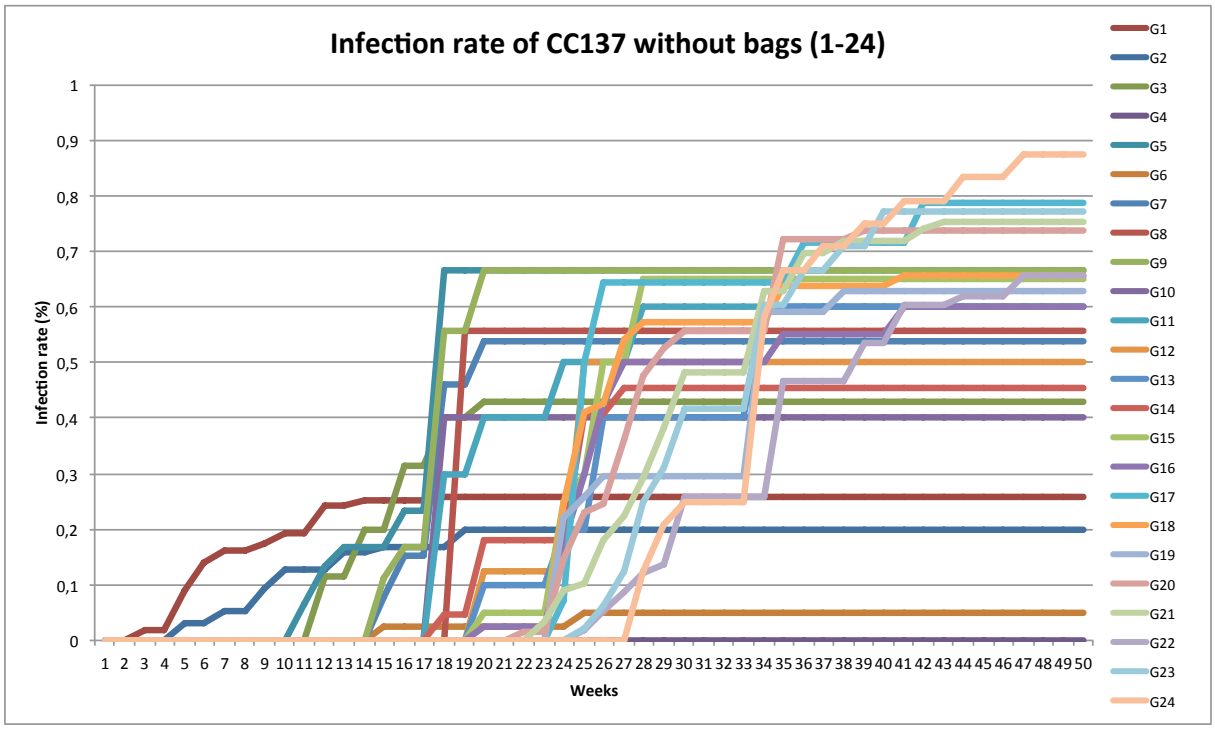


Figure 15. Infection rate of CC137 without bags from generation 1 to 24

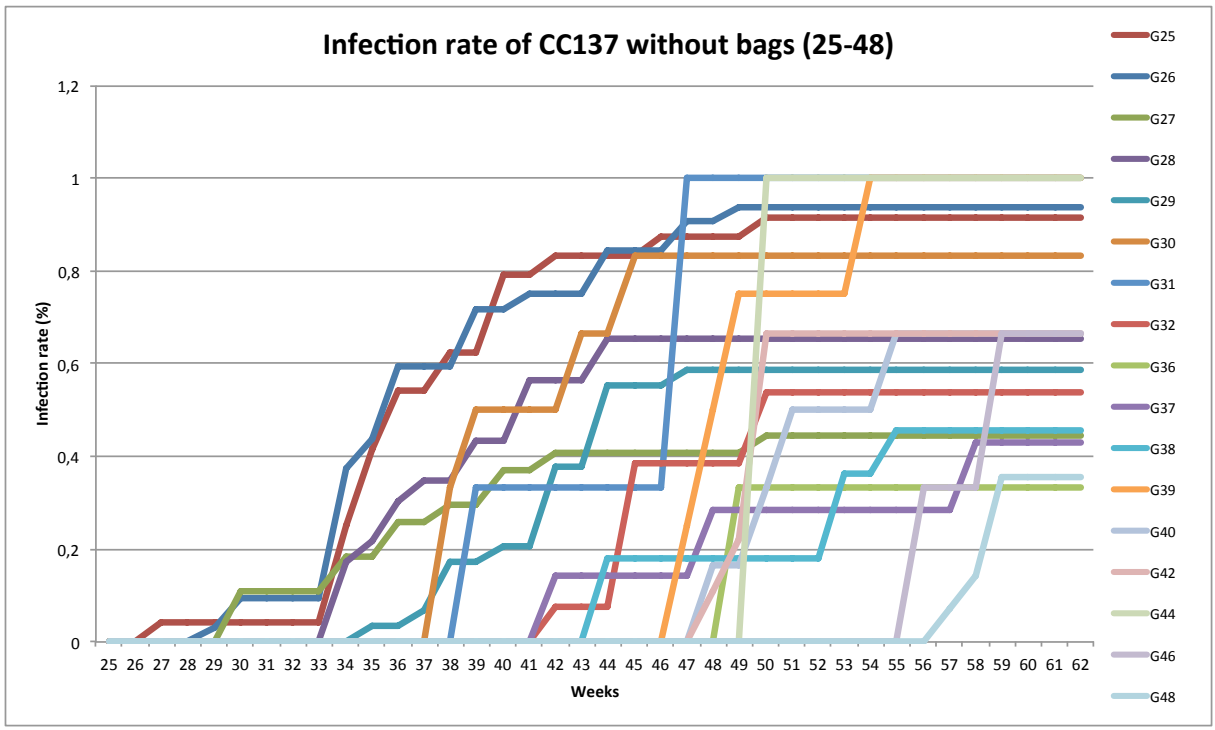


Figure 16. Infection rate of CC137 without bags from generation 25 to 48

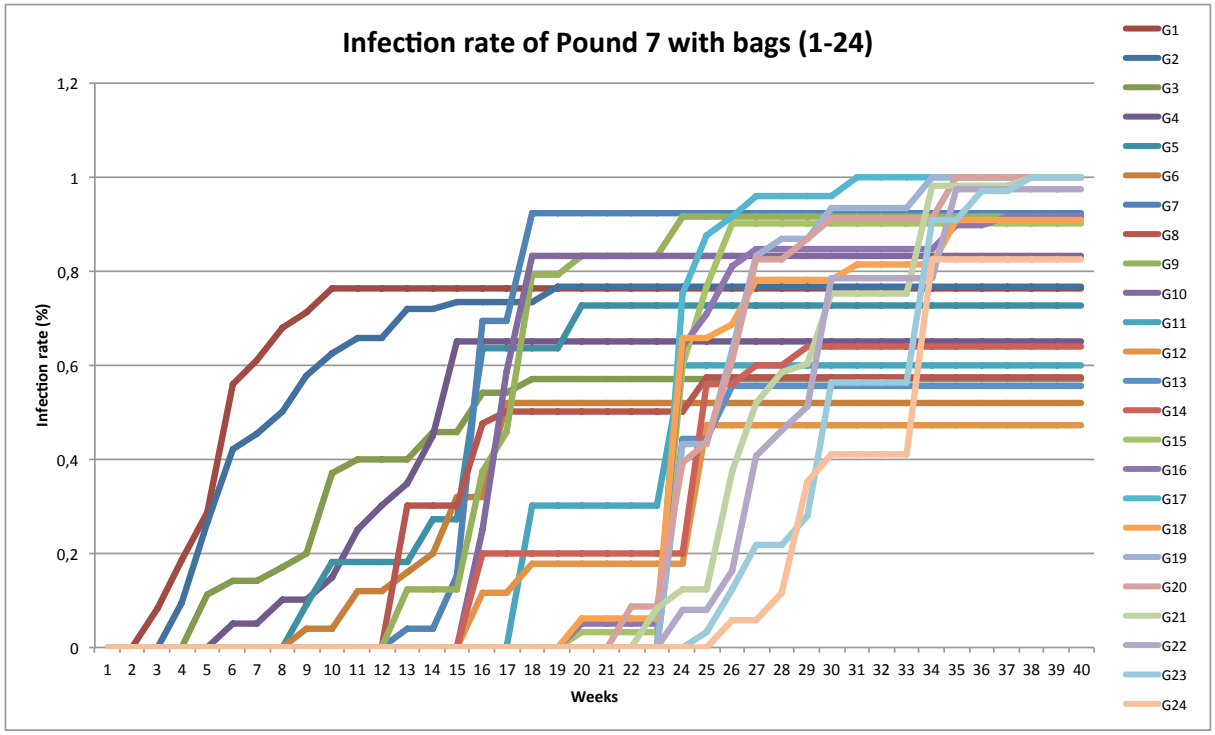


Figure 17. Infection rate of Pound 7 with bags from generation 1 to 24

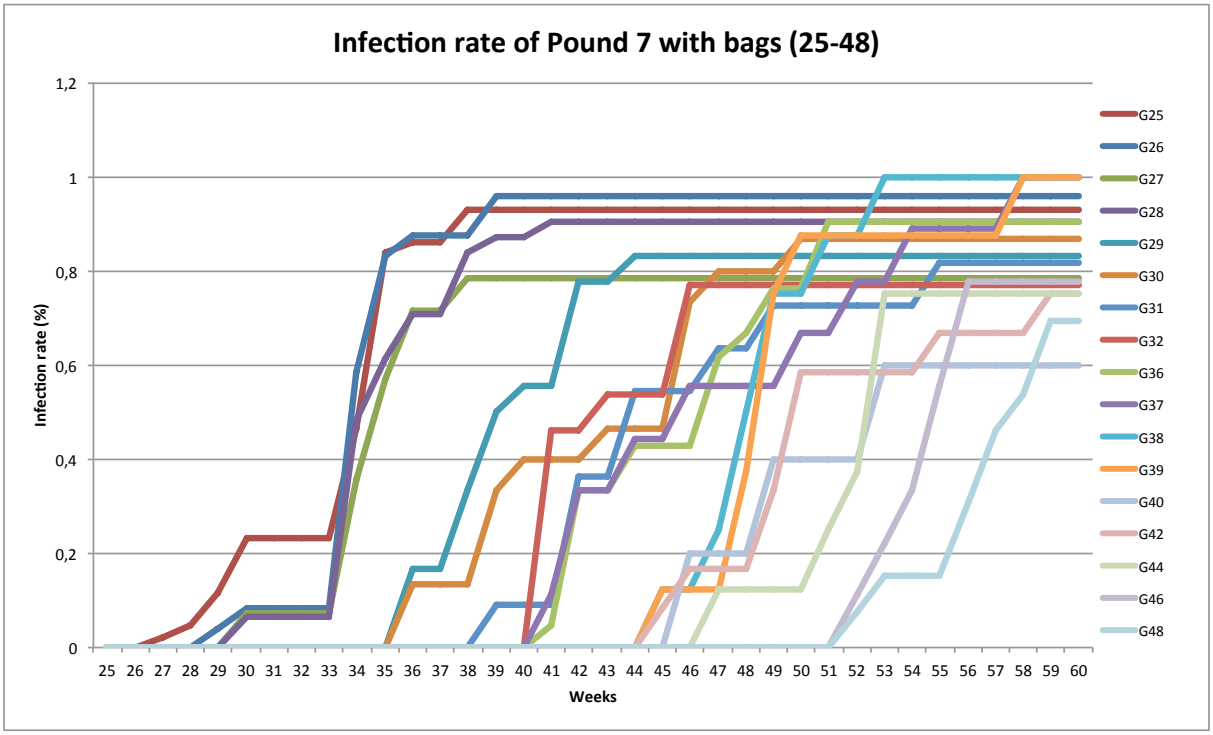


Figure 18. Infection rate of Pound 7 with bags from generation 25 to 48

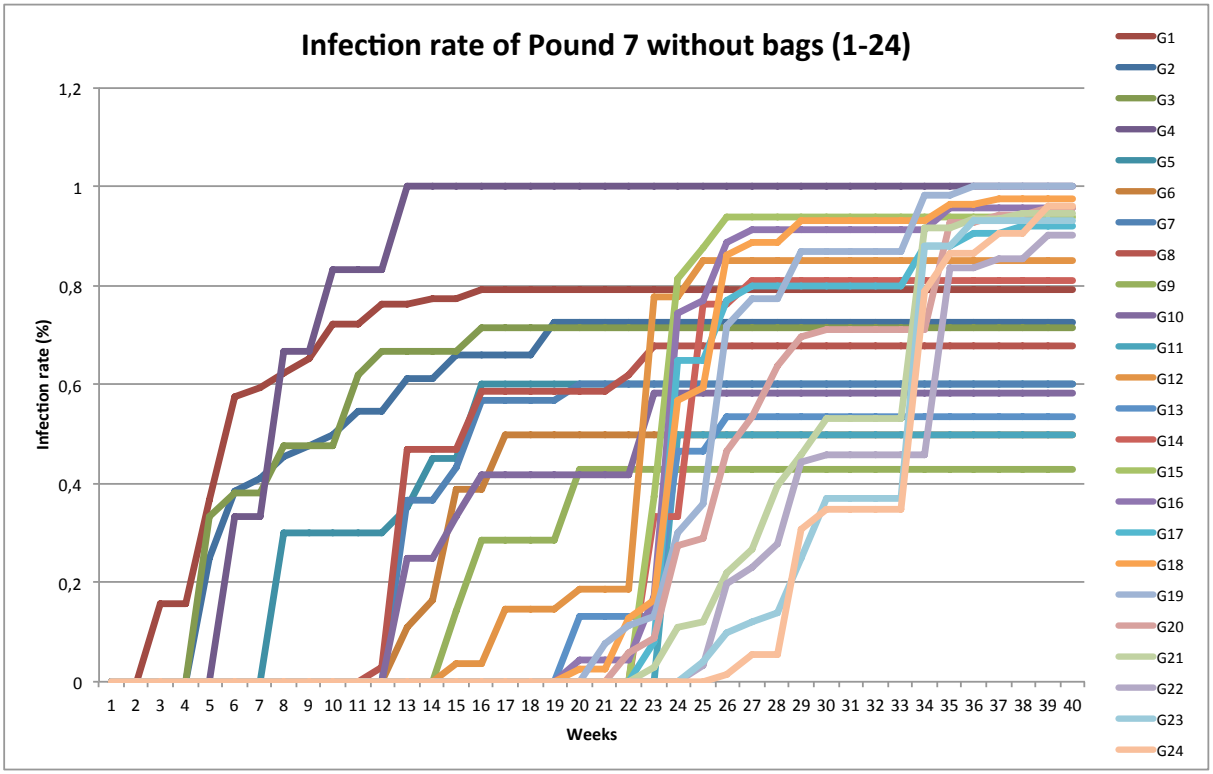


Figure 19. Infection rate of Pound 7 without bags from generation 1 to 24

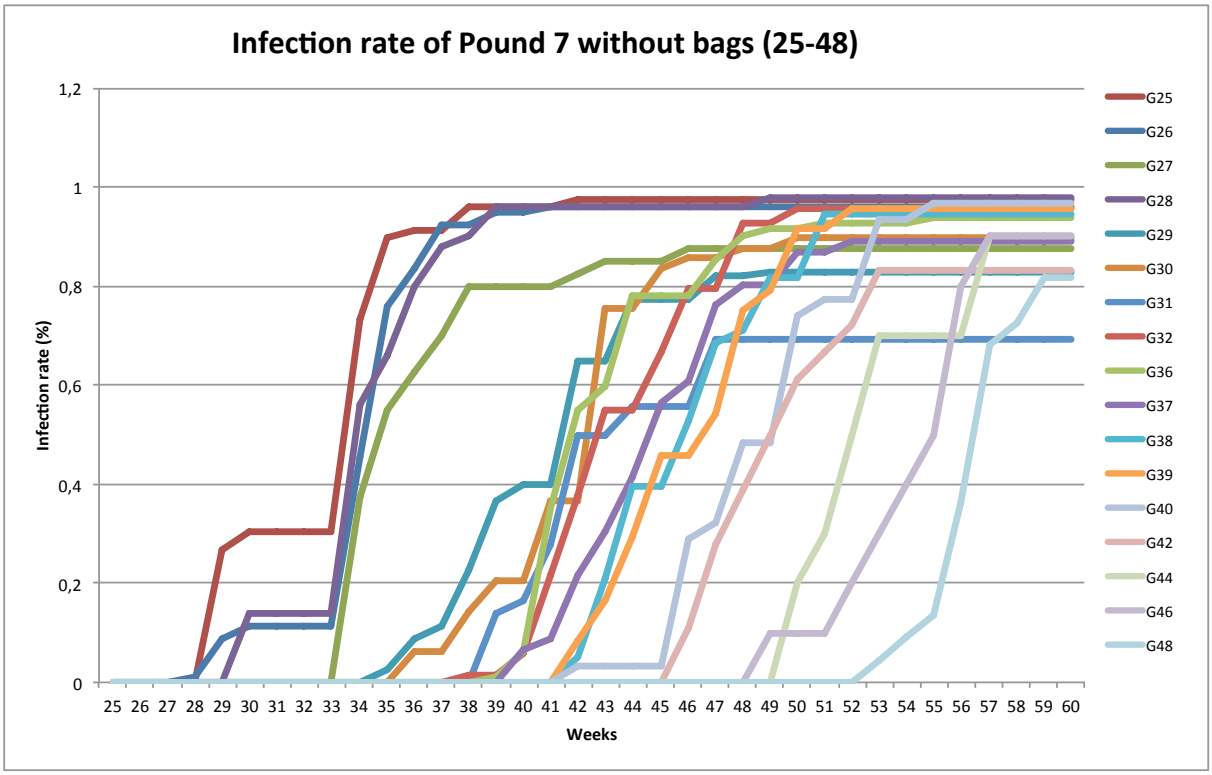


Figure 20. Infection rate of Pound 7 without bags from generation 25 to 48

These graphs confirm that the clones CATIE R4, CC1137 and Pound 7 have different levels of resistance to moniliasis. Pound 7 is the most sensitive and CATIE R4 the most resistant to the disease. CATIE R4 without bags has an average infection rate of 25,8%, whereas Pound 7 has been affected over 83,4% by the disease. But the interaction Clone: Treatment does not have a significant effect (Table 3).

Table 3. Infection rate averages per clone and treatment

Clone and treatment	Infection rate average	p value (Student test)
CATIE R4 with bags	0, 2784071	0, 5983
CATIE R4 without bags	0,2581318	
CC137 with bags	0, 5410962	0, 3224
CC137 without bags	0, 5978899	
Pound 7 with bags	0, 8152646	0, 6041
Pound 7 without bags	0, 8336468	

Generally, the curves have an “inversed J” form, suggesting that moniliasis is a monocyclic disease meaning presenting only one infection stage and followed by symptoms expression brought forward in time. If this hypothesis is true, it means that the disease needs several generations to transmit the fungus in order to the epidemic to progress. Pods removal, which consists in removing diseased pods corroborate this hypothesis because the next generations’ local inoculum is lower and this control does have an impact.

Other curves have an exponential form, meaning that the attack is quick and without symptoms appearance. This difference is due to the fact that some generations were under favourable climatic conditions to the disease when they grew up.

This analyse permits us to appreciate symptoms expression in time. However, it doesn't permit us to explain pods infection. The further analyse aims to explain this infection thanks to climactic factors that occurred before and after life of pods.

Binomial Generalized Linear Model (GLM)

In order to put in relation pods infection and the microclimate, AIC were realized. It permits to evaluate the level of correlation between climatic variables and pods status and to appreciate which are the favourable factors to pods infection – and not to symptoms appearance.

Three periods of time related to the date of labelling pods were tested so that we could put in light the significant date and duration of time during which climate is favourable to pods infection. The periods 0-30 days, 30-60 days and 60-90 days after marking were analysed for daily average temperature, amplitude of temperature and average relative humidity. These periods correspond to about 3, 4 and 5 months of age of pods.

For each AIC graph (Figure 21 to Figure 29), the lower is the AIC (purple point), the better is explained the GLM. However, we didn't take in consideration AIC values located at the extremes of graphs; AIC is estimating the unknown part of the model, so the extremes are values over which we don't have data, so that this AIC could be completely skewed.

CATIE R4

Average temperature

0-30 30-60 60-90

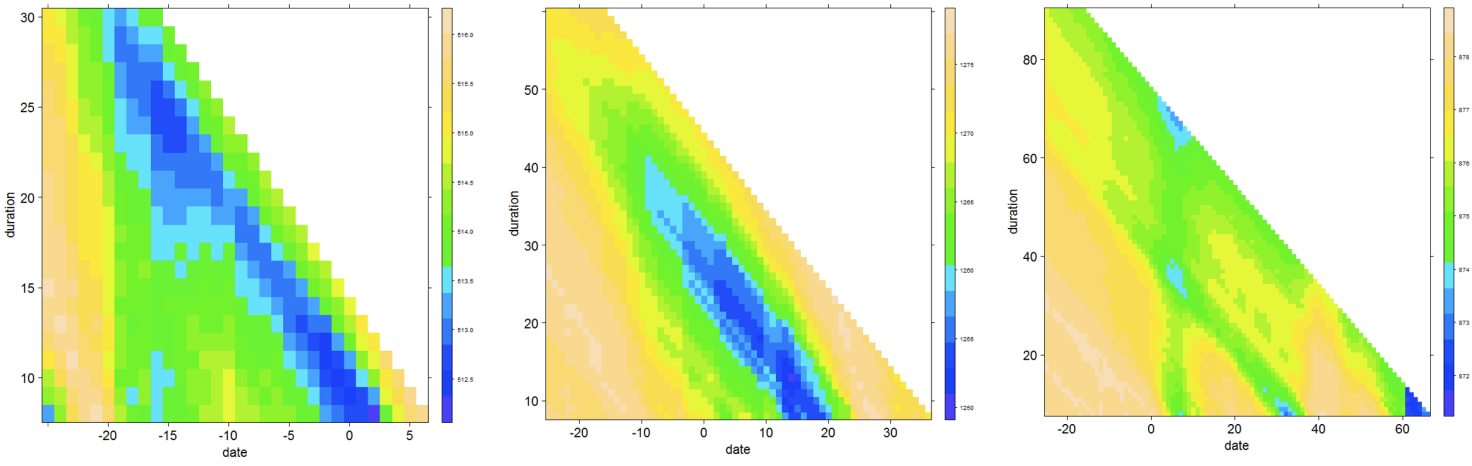


Figure 21. Average temperature for CATIE R4

Daily average temperature doesn't explain well the change of status that occurred in the periods of 0-30 days and 60-90 days after marking because the AIC are located in both cases in the extremes of the axis. But in the period 30-60 days after marking, the average temperature from 13 days after marking and during 13 days explains better the change of status.

Amplitude of temperature

0-30 30-60 60-90

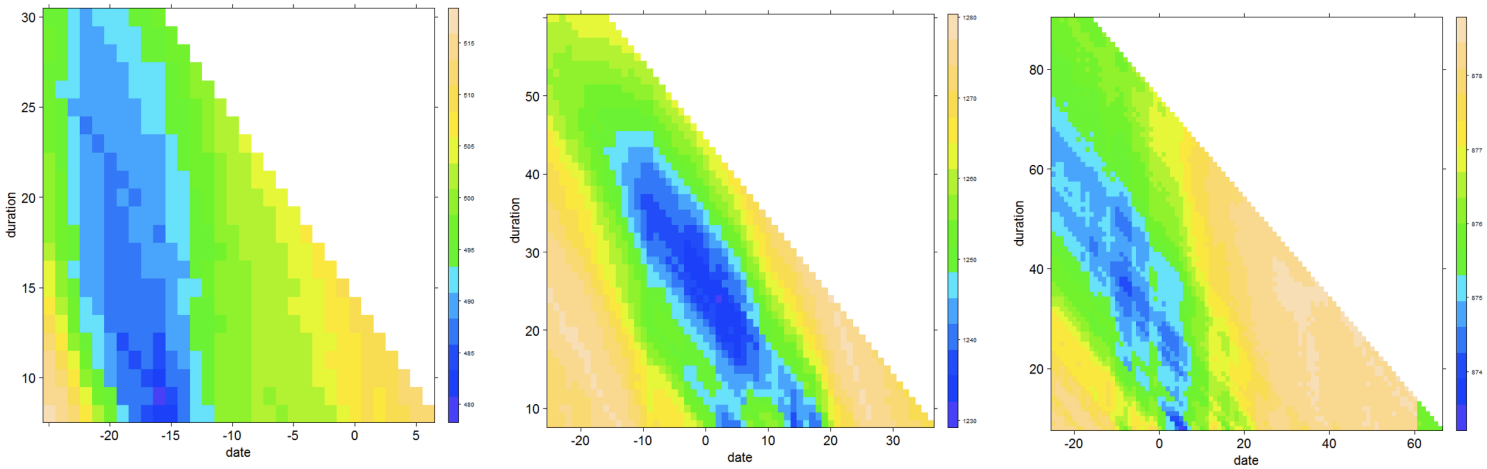


Figure 22. Amplitude of temperature for CATIE R4

According to the graphs of amplitude of temperature, 15 days before marking and during about 6 days the amplitude of temperature influences the most significantly change of status, which will be observed 60 days after marking. Again, this period of observation 30-60 days after marking is the best of the three.

Average relative humidity

0-30 30-60 60-90

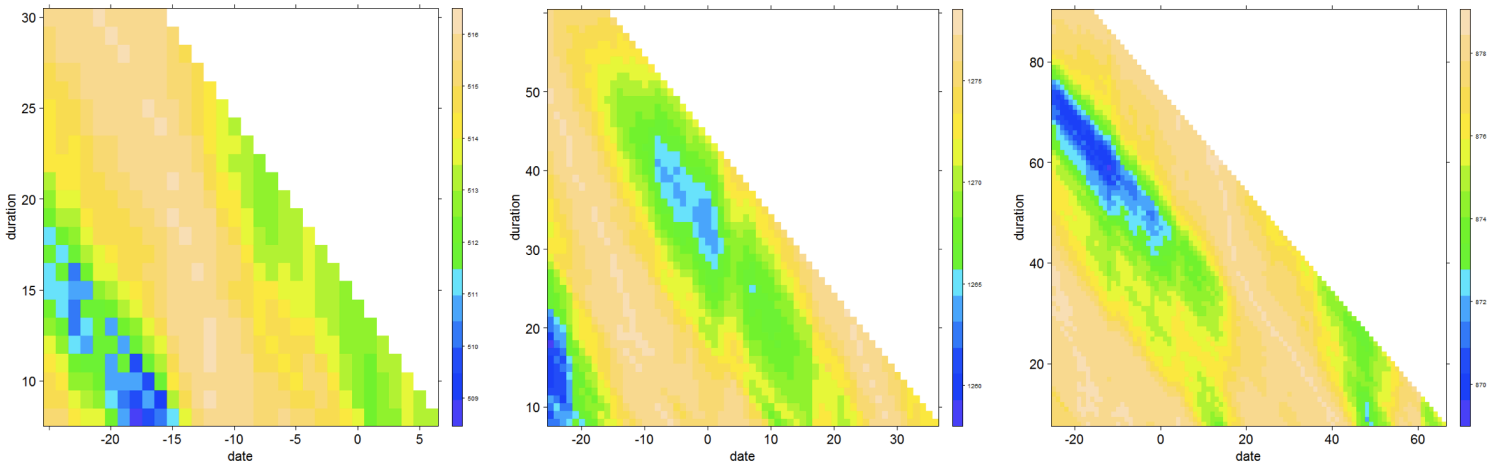


Figure 23. Average relative humidity for CATIE R4

According to the graphs of daily average relative humidity, about 12 days before marking and during 60 days, average relative humidity influences the most significantly the change of status observed from 60 to 90 days after marking. It seems that average relative humidity 7 days before marking and until 1 day after during a period of 31 to 38 days also influences the change of status, but between 30 to 60 days after marking. However, this latter period of time is less significant than the first.

We notice that in the period 60 to 90 days after marking, only relative humidity is well explained by the prior climate. Changes of status are globally observed 30 to 60 days after marking, meaning at about 4 months of age of the pods.

CC137

Average temperature

0-30 30-60 60-90

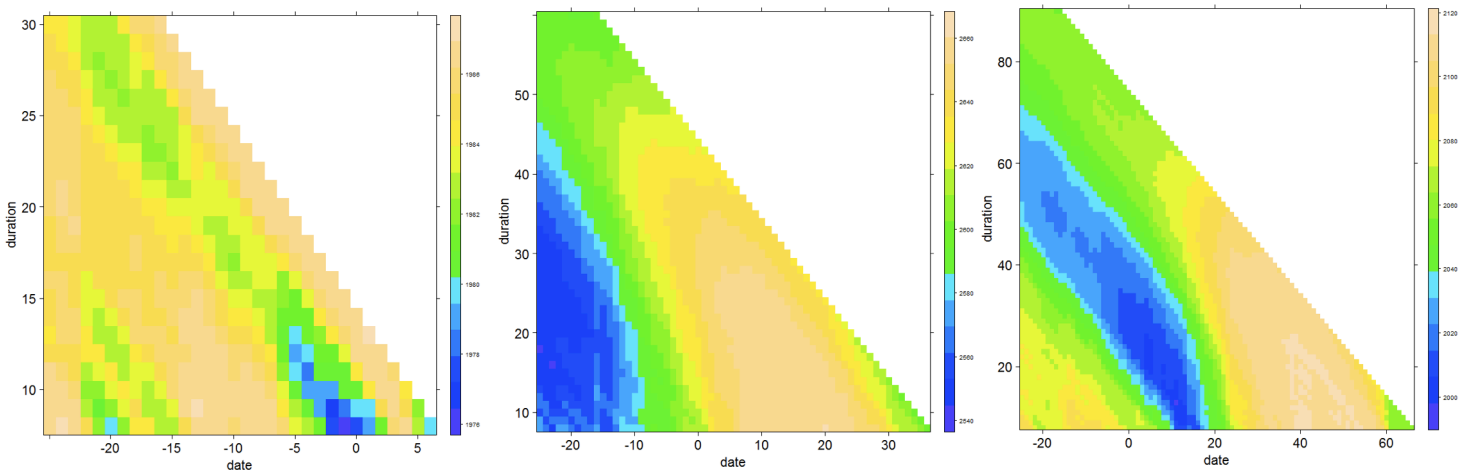


Figure 24. Average temperature for CC137

During the periods 30-60 days and 60-90 days after marking, daily average temperature that triggered the change of status of pods is well explained. Pods infection that occurred on the period 30-60 days is explained by average temperature arose 23 days before during 16 days. However, in the period 60-90 days, it is average temperature that occurred 11 days after marking and during 12 days that explains pods infection.

Amplitude of temperature

0-30 30-60 60-90

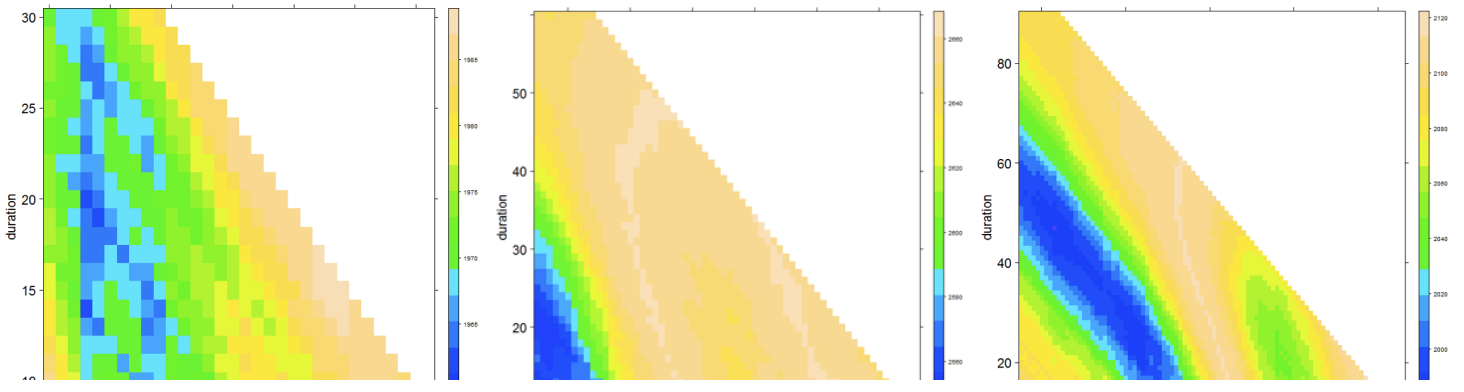


Figure 25. Amplitude of temperature for CC137

Amplitude of temperature that triggers infection is best explained in the period 60-90 days after marking. The amplitude of temperature at 16 days before marking during about 46 days influenced most the change of status observed in the period 60-90 days.

Average relative humidity

0-30 30-60 60-90

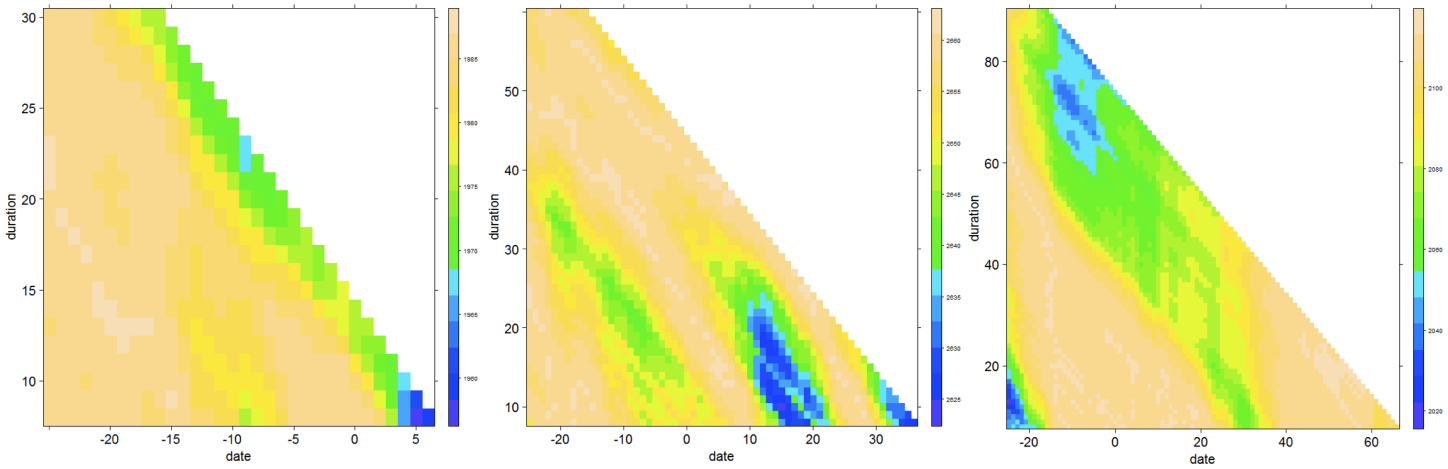


Figure 26. Average relative humidity for CC137

During the period of observation 30-60 days after marking the AIC of average relative humidity is best represented. Relative humidity that occurred 15 days after marking and during 10 days arose the change of status of the pods.

Pound 7

Average temperature

0-30 30-60 60-90

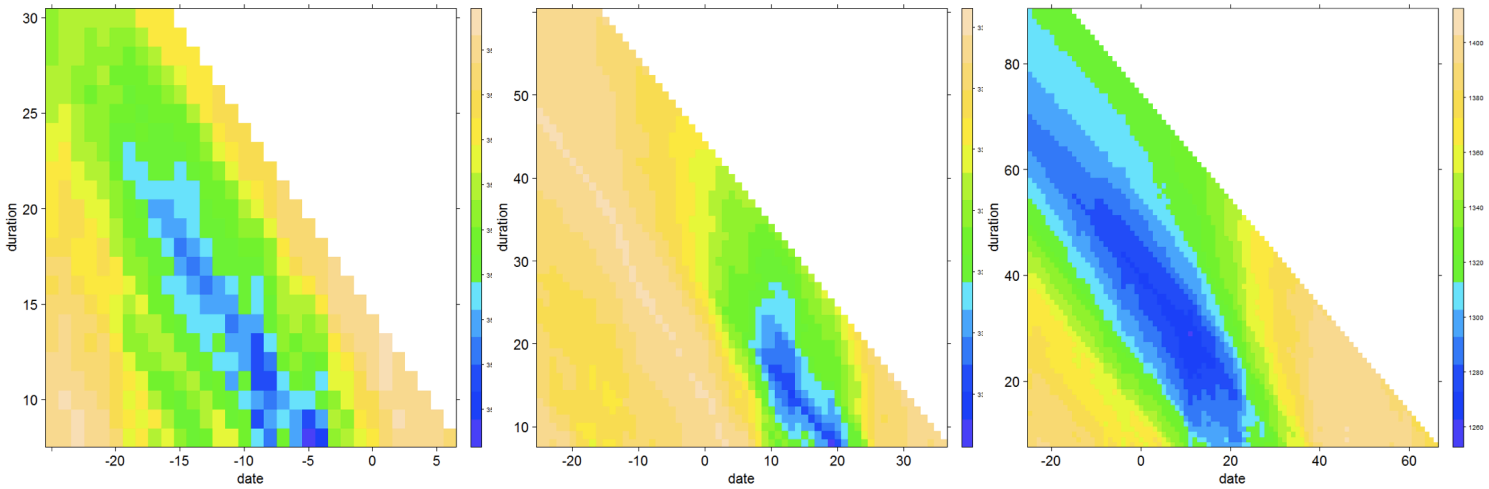


Figure 27. Average temperature for Pound 7

The best AIC value is obtained in the period 60-90 days after marking. About 10 days after marking and during 28 days average temperature influences the most the change of status of pods of Pound 7.

Amplitude of temperature

0-30 30-60 60-90

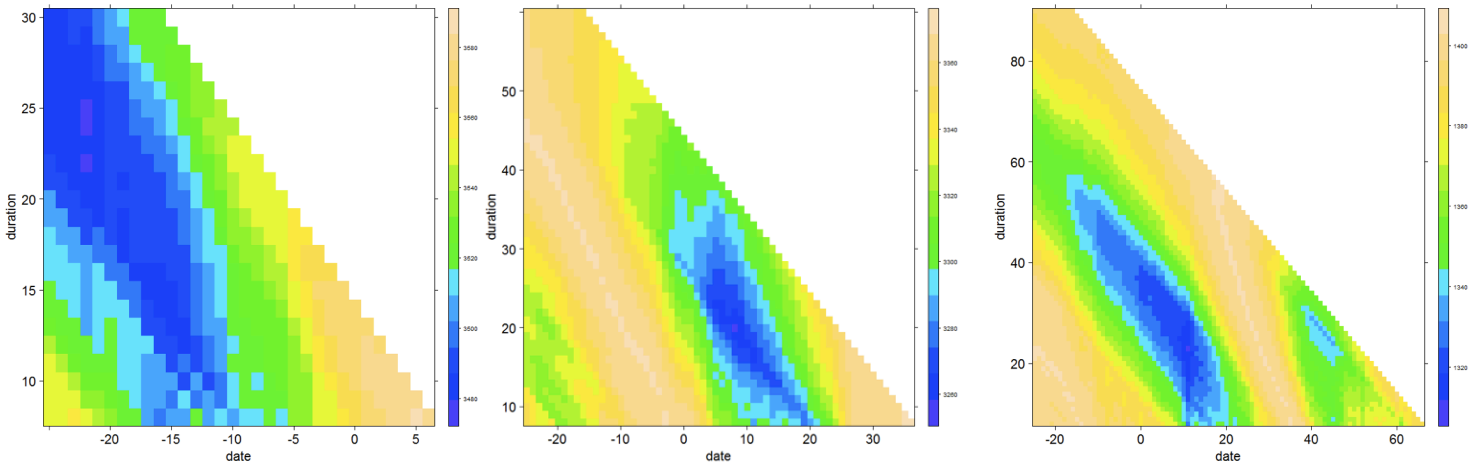


Figure 28. Amplitude of temperature for Pound 7

The amplitude of temperature that triggers a change of status of pods is well explained in all periods. For the period 0-30 days after marking, the key amplitude occurred 13 days before marking during 25 days. For the periods 30-60 days and 60-90 days after marking, we obtained the same intervals in both periods: amplitude of temperature that occurred 8 to 12 days after marking during 19 to 25 days influenced the change of status.

Average relative humidity

0-30 30-60 60-90

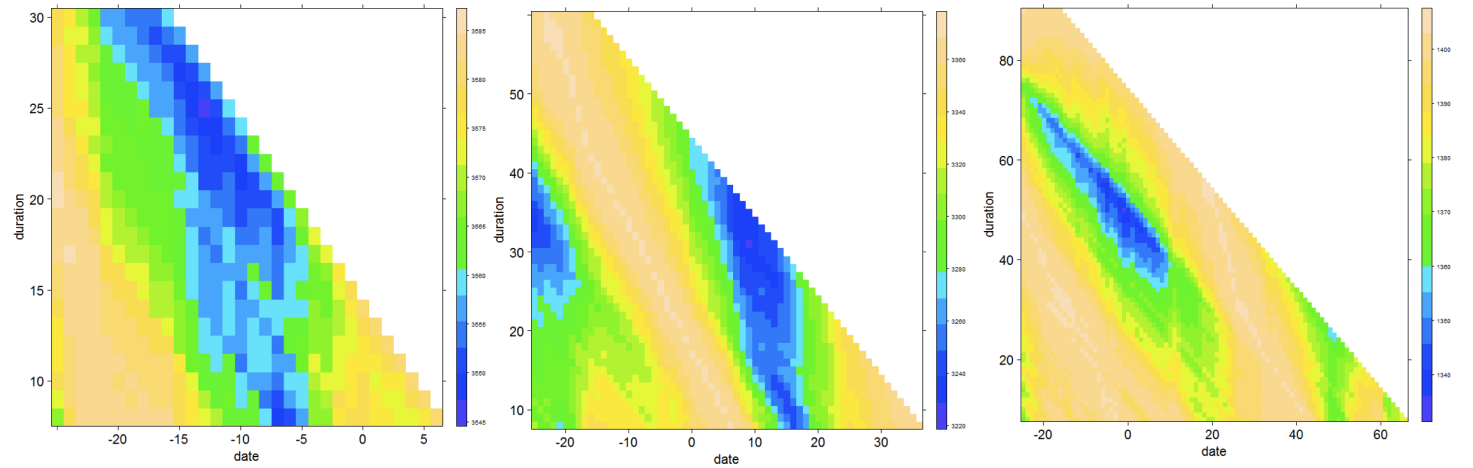


Figure 29. Average relative humidity for Pound 7

The best AIC representation for relative humidity that influenced the change of status is observed in the period 60-90 days 1 day before marking and during 50 days.

In Figure 30 are summed up the key periods for each climatic variable that influences the change of status observed in the three studied periods 0-30, 30-60 and 60-90 days after marking (Annexe 2).

First of all, it highlights that for one studied period after marking, the clones react very differently to climatic conditions: very few key periods of action of the climatic variables are similar between the clones, excepted maybe for the period 60-90 days after marking. For the studied period 0-30 days after marking, the clone CC137 does not have any key period because the AIC representations were very bad for each variable.

We also noticed that environmental conditions influenced on the change of status from the very birth of the pod (- 20 days represents about 1 month of age). And this is an important point to underline for the crop protection.

The variable of precipitation was included in this first model; however results did not give any valid value (bad representations of AIC for every clone).

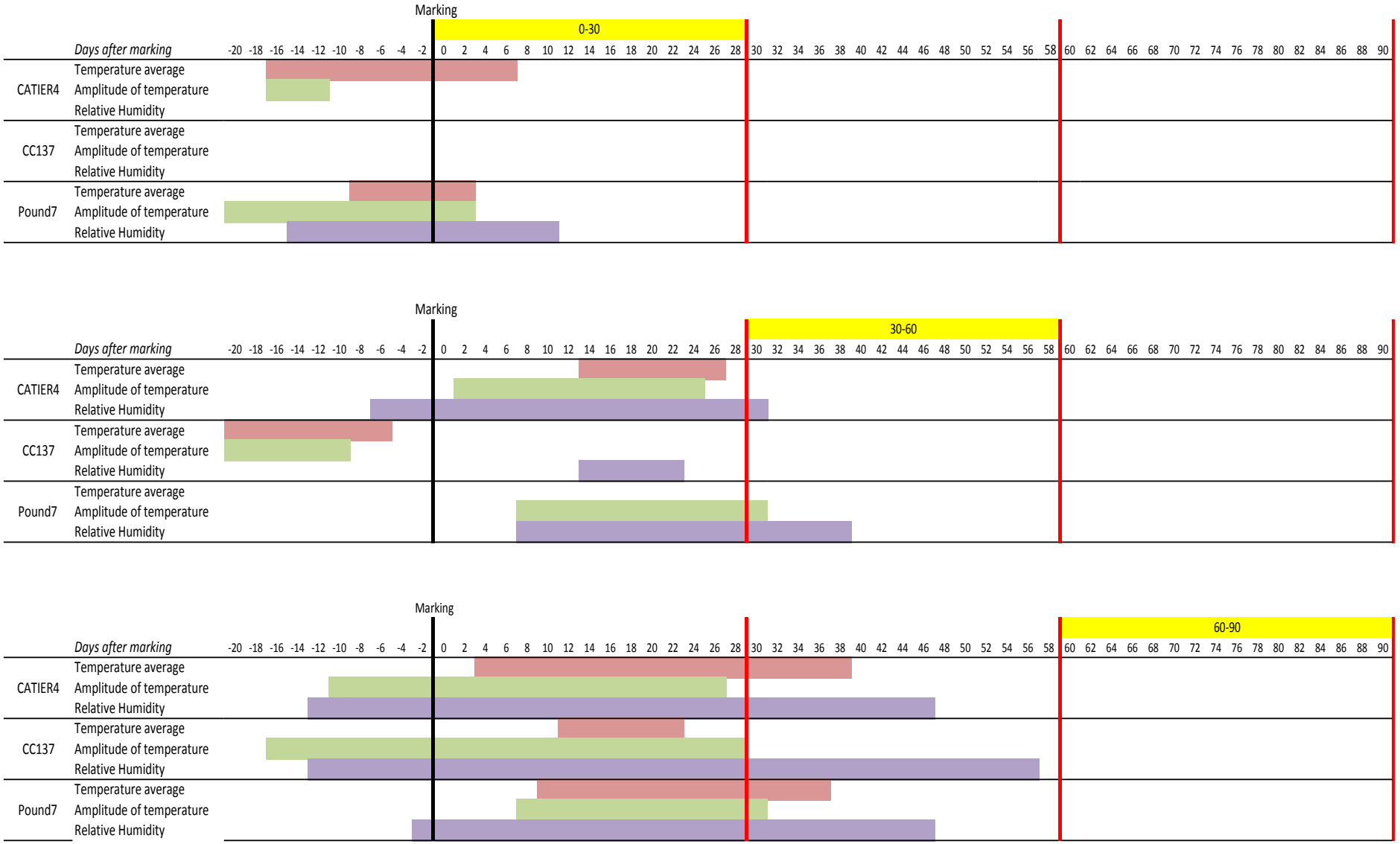


Figure 30. Period of effect of each climatic variable on the probability of the pods to be infected during the studied period

Complete binomial Generalized Linear Model

The continuation of this first analysis of climatic variables in relation with pods infection is the construction of another generalized linear model. It tests intervals of interest from the previous analysis and including the variables of precipitation and moisture for each clone and treatment. The further example illustrates the added value of this model. A complete binomial model for the probability of infection of Pound 7 with and without bags during 60 and 90 days after marking was tested as an example.

During this period, the average temperature beginning 11 days after marking and during 29 days is different from the others and explains the change of status (Figure 31). The amplitude of temperature beginning 11 days after marking and during 21 days explains the change of status in the period too (Figure 32) and the average relative humidity beginning 1 day before marking and during 50 days explains the change (Figure 33).

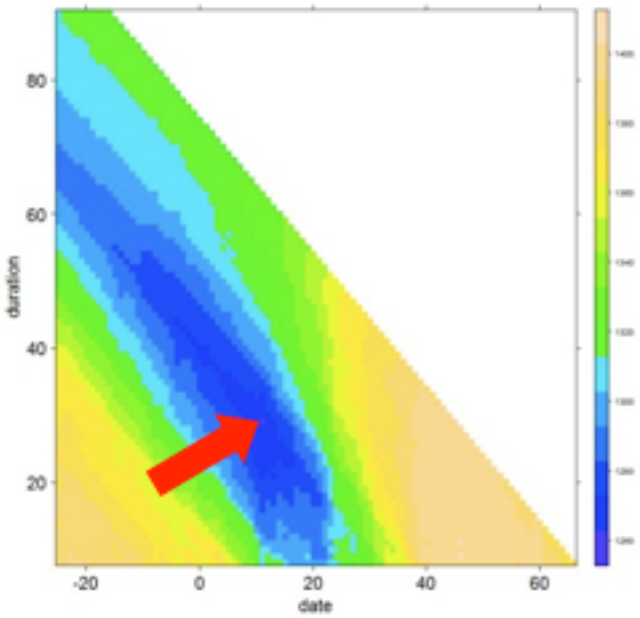


Figure 31. Average temperature for Pound 7 in complete GLM

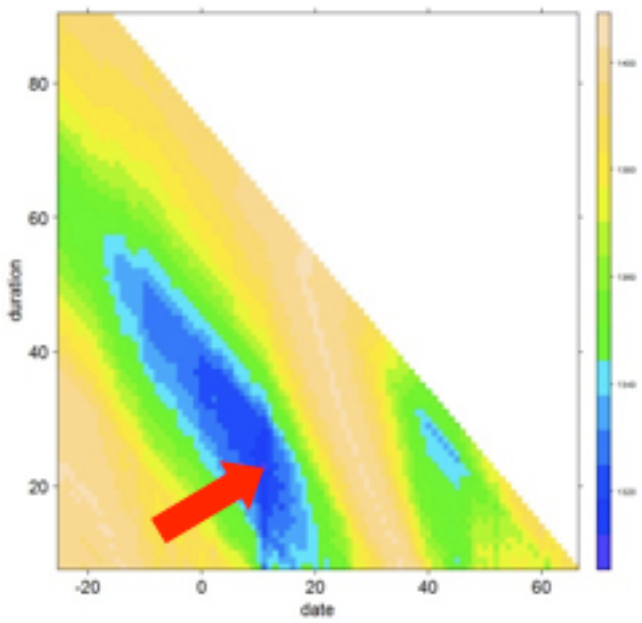


Figure 32. Amplitude of temperature for Pound 7 in complete GLM

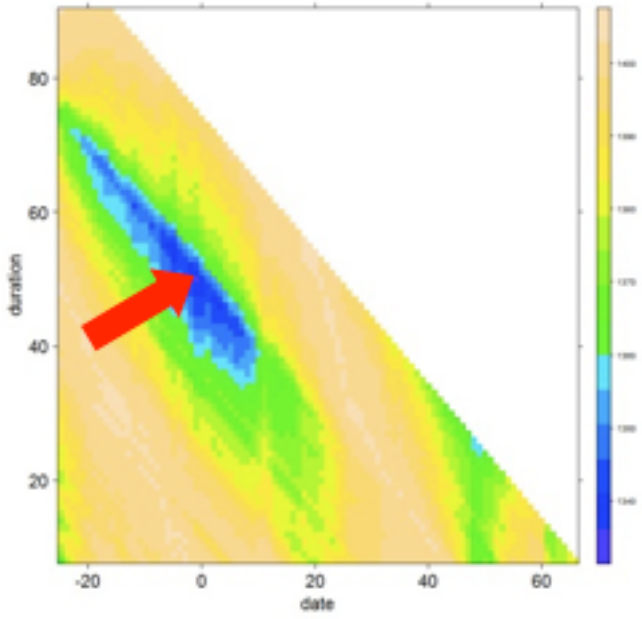


Figure 33. Average relative humidity for Pound 7 in complete GLM

For each of these latter intervals, the model creates a graph of the probability of infection for a range of average temperature and amplitude of temperature, and for a relative humidity equal to 80, 90 and 100% (Figure 34).

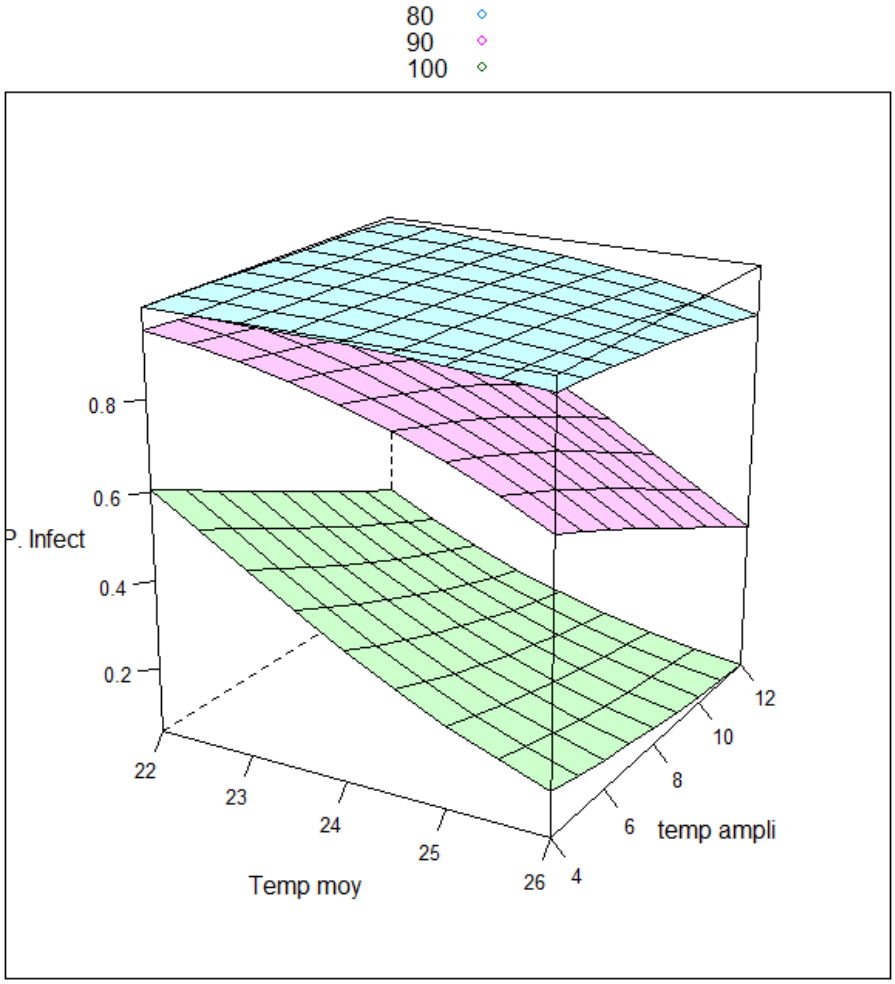


Figure 34. Probability of infection for a range of mean temperature and amplitude of temperature, and for a relative humidity equals to 80, 90 and 100% for Pound 7

We created this model for relative humidities equal to 80, 90 and 100% because it is known that the fungus thrives with high relative humidities. According to the model, a relative humidity equal to 80% involves an infection rate close to 100%. And the more relative humidity is higher, the more the infection rate decreases. We also notice that high amplitudes of temperature are not favourable to the fungus development.

In reality, on the field, we observe low amplitudes of temperature when it did not rain and the weather was hot all day long. So average temperature is high and according to the model, infection rates are lower than when average temperature is low. The model suggests that at "low" average temperatures, the infection rate is higher. But Leandro-Muñoz (2011) demonstrated that the optimum of temperature of the fungus is included between 24 and 28°C. Observing a high infection rate at low temperatures cannot be observed on the field. On the field we really observe high amplitude of temperature because of the rain, and the rain involves a decrease of temperature. Average temperature for these days is consequently low.

And inadequate conditions are observed with high amplitudes of temperature, low relative humidity and high averages temperature.

Discussion and conclusion

Factors effects on change of status of pods at removal date

The analysis of the factors influencing the change of status of pods (transition from healthy to diseased) shows that, beyond the clone, the generation is decisive because interactions with this factor are all significant. Thus, CC137 can reach infection rates close to those of CATIE R4 or Pound 7, although CATIE R4 and Pound 7 are very different in term of resistance to monilia. And every interaction with the generation is significant; this underlines that the generation is crucial. It means that at some times, there is a great monilia pressure and at some other times this pressure is low. This result suggests being more interested in cocoa phenology in programs of selection to select clones more productive in period of non-infection by moniliasis. So that it would permit to escape from the disease. This would represent a trail to study for moniliasis control.

However, even if almost every factor and interaction is significant, some effects are negligible and they are not worth the effort to be taken into account. This high level of significance is due to the great number of data.

Infection rate of each clone by moniliasis

The comparison of each clone according to the treatment confirmed the differences of resistance between our clones. CATIE R4 is the most resistance and Pound 7 the most sensitive. These results confirm the previous results on the differences of resistance of CATIE R4, CC137 and Pound 7 (Phillips-Mora *et al*. 2012).

Bagging the pods did not show any significant difference, but on the field, this practice is the only way known and effective to date. Currently, the only disease control known and efficient is the pods removal based on regular observation of pods in order to eliminate as soon as possible symptomatic pods. And this disease management must be carried on. Despite this weighty control (it must be a weekly control and diseased pods must be buried to prevent from spores dispersion), this disease management is curative and does not permit to foresee the disease. Once pods show symptoms it is too late, hence the interest of highlighting the key climatic variables in pods infection.

Maybe the local inoculum must be studied at the plot scale and around the plot because it is obvious that eliminating it only on the plot is insufficient.

Binomial Generalized Linear Model (GLM)

For clone CATIE R4, the variables of average temperature and amplitude of temperature explain best the changes of status occurred during the period of observation 30-60 days. Average relative humidity influences changes of status between 60 to 90 days after marking.

For clone CC137, the average temperature and average relative humidity explain the changes of status occurred during the period of observation 30-60 days. The amplitude of temperature influences more changes occurred after 60 days of observation.

Climatic variables that occurred during the period of observation 60-90 days for Pound 7 explain best the changes of status.

The observation of the summary table of dates and key periods of the climatic variables influencing the changes of status highlights that the variables influencing each clone do not overlap (Figure 30). These differences between clones and particularly between periods observed are not very clear. Other analyses must be carried on, and especially by integrating the variable of precipitation in the model because it plays a great part in spores' dispersion and the disease development. The period that explains best the effects of the climate is 30 to 60 days after marking. But dates and durations during which climatic variables influence on infection are very different. And this highlights again the interest to build more complete models. Theses results remain explorative for the moment and show that it must be carried on to identify key factors in moniliasis development. The AIC intervals here obtained should permit to select other intervals more accurate.

Maybe this high variability can be explained by the fact we labelled only healthy pods in appearance. But clone Pound 7 shows very quickly symptomatic pods because of its sensitivity to moniliasis. Thus these pods were not labelled, whereas for the two other clones, which can have diseased pods but healthy in appearance. So we eliminated from the beginning pods that could have expressed symptoms forward in time, maybe in periods 0-30 or 30-60 days for clone Pound 7.

As regards to cocoa phenology a study must be carried out. A better understanding of factors influencing the disease development and a work on cocoa phenology should allow us to improve management strategies for this disease.

Moreover, the model was tested without distinction of the treatment (bags/without bags) because first analyses gave a non-significant effect of the treatment (analysis realized with less pods).

Complete binomial Generalized Linear Model

As explained in the complete GLM analyse, we must interpret the results of the complete model with caution: the model gives for each relative humidity a theoretical infection rate. However, at given relative humidity, when the amplitude of temperature is high, the temperature is not high.

This model is explorative for the moment, and its results, according to its curves, are not always true on the field.

New leads

Further to this explorative work on the effects of the pod production dynamic of the cocoa tree and inoculum sources on the FPR development, some new leads are to be studied more accurately.

Concerning the complete binomial GLM, the variable of precipitation should be added. Precipitations play an important role in the fungus development. Because of lack of data and of time, we did not include this variable to the models. But it is obvious that this variable is of very importance.

The correlation between climatic variables is complex. The model does not underline which one is the most influent, if one is really the most influential. In order to test this hypothesis, inoculations in controlled conditions should be realized.

We realized a linear model but it is possible that the relations between our variables are not linear. So that the model should be improved, including first of all the variable of precipitation. For that, we aim to identify the moisture threshold above which vegetation and pods are wet. When this threshold is exceeded, we suppose that the probability of infection increases significantly because rain and relative humidity are favourable factors to the fungus.

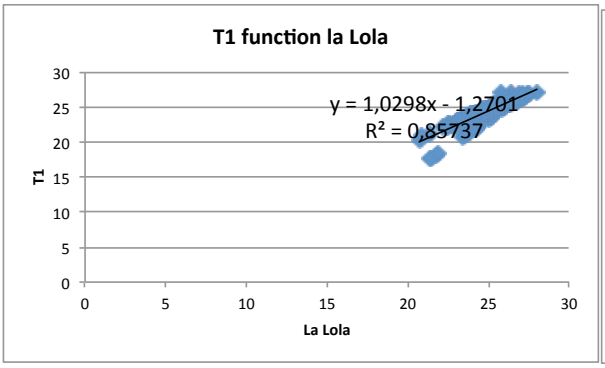
We should also run again the analysis separating the treatments because we finally observed a significant difference between clones with and without bags.

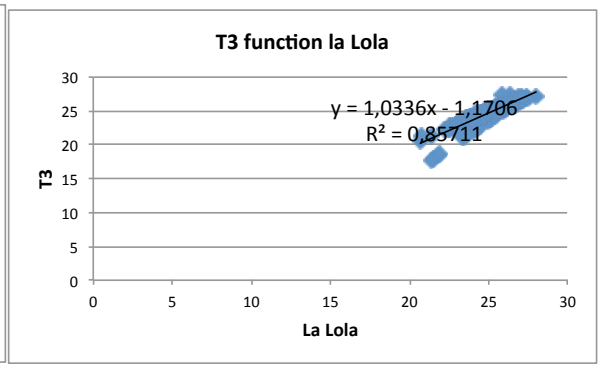
Concerning the local inoculum, maybe it must be studied at the plot scale and around the plot because it is obvious that eliminating it only on the plot is insufficient. And studying cocoa phenology should permit to highlight interesting results on a way to escape from the disease.

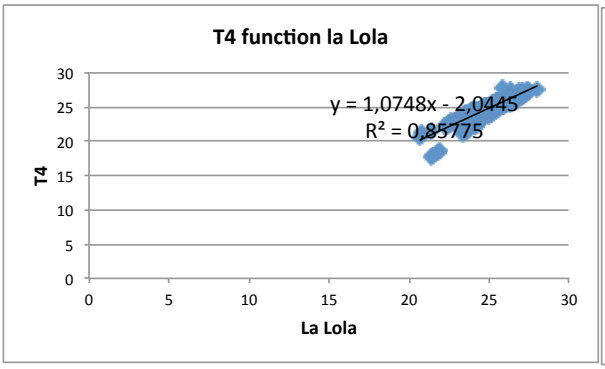
In conclusion, although we highlighted interesting results on favourable climatic conditions to monilia development for clones with different resistances, this work remains explorative and we need to define with precision the variables and especially key periods playing a part in pods infection. This work introduces the bases of another model.

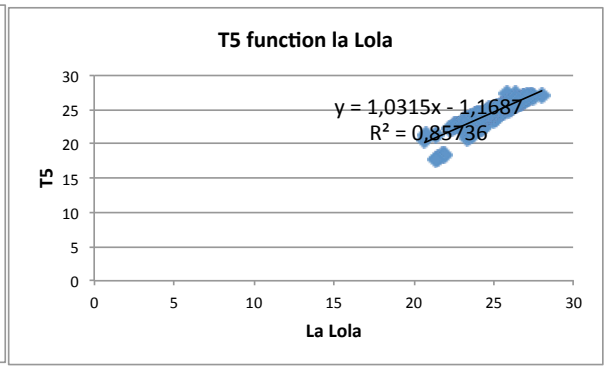
Annexes

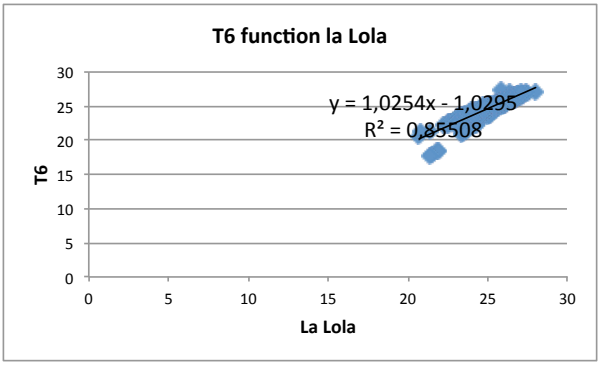
Annexe 1. Microclimate calibration curves

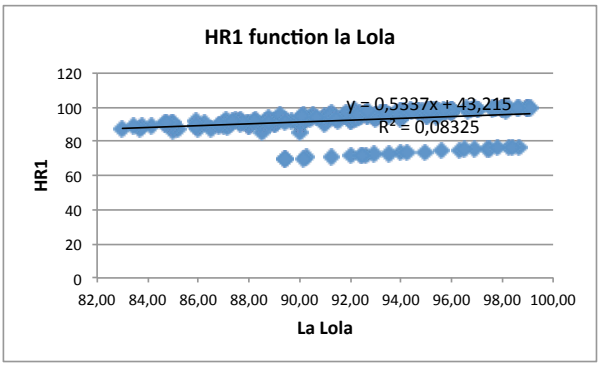


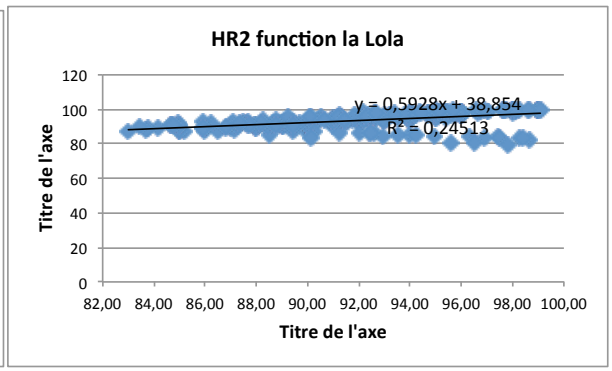












Annexe 2. Key periods for each climatic variable that influences the change of status observed in the three studied periods 0-30, 30-60 and 60-90 days after marking (number of days of influence) for each clone

CATIER4		Period		
		0-30	30-60	60-90
Mean temperature	Date	[-17;-14]	[13;14]	[4;7]
	Duration	[24;26]	[12;14]	[35;37]
Amplitude of temp	Date	[-16;-15]	[2;5]	[-11;-7]
	Duration	[5;7]	[23;25]	[35;38]
Relative Humidity	Date	[]	[-7;1]	[-13;-12]
	Duration	[]	[31;38]	[59;60]

CC137		Period		
		0-30	30-60	60-90
Mean temperature	Date	[]	[-24;-22]	[11;12]
	Duration	[]	[15;17]	[12;13]
Amplitude of temp	Date	[]	[-24;-23]	[-17;-16]
	Duration	[]	[11;13]	[46;47]
Relative Humidity	Date	[]	[14;16]	[-12;-10]
	Duration	[]	[9;11]	[71;73]

Pound7		Period		
		0-30	30-60	60-90
Mean temperature	Date	[-9;-8]	[]	[10;12]
	Duration	[11;13]	[]	[28;29]
Amplitude of temp	Date	[-22;-21]	[8;12]	[8;12]
	Duration	[19;25]	[19;25]	[19;25]
Relative Humidity	Date	[-14;-12]	[8;9]	[-2;0]
	Duration	[24;26]	[30;32]	[49;51]

References

Anonymous. 1832. El cacao. *El Cultivador Cundinamarqués o Periódico de la Industria Agrícola y de la Economía Doméstic*a, no. 7, 1 de mayo de 1832.

Anonymous. 2006. Informe mensual del avance de la moniliasis del cacao en Tabasco. Villahermosa, Tabasco, México: CESVETAP-SAGARPA, Oficina de la Subdelegación de la SAGARPA.

Aranzazu F. 2000. Escoba de bruja en Colombia : su impacto económico y manejo. Tecnología para el mejoramiento del sistema de producción de cacao. In: Mejia FLA and Arguello CO, eds CORPOICA, Bucaramanga, Colombia.

Barros O. 1980. El control de la moniliasis en Cacaoteras del Dique. *El Cacaotero Colombiano.* 15:31-44.

Bartley BGD. 2005. *The Genetic Diversity of Cacao and its Utilization*. CABI Publishing. Wallingford, UK.

Beer J, Muschler R, Kaas D & Somarriba E. 1998. Shade management in coffee and cocoa plantations. *Agroforestry Systems*. 38:139-164.

Cubillos G & Aranzazu F. 1979. Comparacion de tres frecuencias de remocion de frutos enfermos en al control de Monilia roreri. *El Cacaotero Colombiano* 8 :27–34.

Desrosiers R & Díaz J. 1957. The world distribution of diseases of cacao. In : *Proceedings of the Sixth Meeting of the Inter-American Technical Committee of Cacao*, Salvador, Brazil. Pages 331-344.

Enríquez GA, Brenes O & Delgado JC. 1982. Desarrollo e impacto de la moniliasis del cacao en Costa Rica. Proceedings of the Eighth International Cocoa Research Conference, 1981. Cocoa Producers' Alliance, Cartagena, Colombia.

Evans HC. 1973. New developments in black pod epidemiology. *Cocoa Growers' Bulletin*. 20:10-16.

Evans HC. 1981a. Pod rot of cacao caused by *Moniliophthora* (*Monilia*) *roreri*. *Phytopathology Papers*. 24 :1-44.

Evans HC. 1981b. Witches' broom disease—A case study. *Cocoa Growers' Bulletin*. 32:5-19.

Evans HC. 1986. A reassessment of *Moniliophthora* (Monilia) pod rot of cocoa. *Cocoa Growers' Bulletin*. 37:34-43.

Evans HC, Krauss U, Ríos-Ruíz R, Zecevich-Acosta T & Arévalo-Gardini E. 1998. Cocoa in Peru. *Cocoa Growers' Bulletin*. 51:7- 22.

Evans HC. 2007. Cacao disease – Trilogy trilogy revisited. *Phytopathology* 97:1640-1643.

Hawker L E. 1950. *Physiology of fungi*. London, University of London Press. 360 p.

Herrera F. 1988. Efecto de factores nutricionales y físicos sobre el crecimiento y esporulación de *Moniliophthora roreri in vitro*. Thesis Master of Science. Turrialba, Costa Rica. Universidad de Costa Rica. 159 p.

Holdridge LR. 1967. *Life Zone ecology*. Tropical Science Center, San Jose, Costa Rica. 206 pages.

ICCO Annual Report 2010/2011. 2012. [html pages]. http://www.icco.org/about-us/icco-annual-report.html. Visited 21 July 2013.

Judd WS, Campbell CS, Kellogg EA, Stevens PF & Donoghue MJ. 2002. Plant Systematics. A Phylogenetic Approach. 2nd ed. Sinauer Associates, Inc., Sunderland, MA.

Krauss U & Soberanis W. 2001. Rehabilitation of diseased cacao fields in Peru through shade regulation and timing of biocontrol measures. *Agroforestry Systems*. 53:179-184.

Lanaud C, Motamayor JC & Sounigo 0. 2003. In : Hamon P, Seguin M, Perrier X & Glaszmann JC, *Genetic Diversity of Cultivated tropical Crops*. Eds Montpellier : CIRAD, Montpellier. 125-156.

Leach AW, Mumford JD & Krauss U. 2002. Modelling *Moniliophthora roreri* in Costa Rica. *Crop Protection*. 21:317-326.

Leandro-Muñoz ME. 2011. Efecto de los factores macro y microclimáticas y las características productivas del cacao sobre la epidemiología de la moniliasis. Thesis Master of Science. Turrialba, Costa Rica. Centro Agronómico Tropical de Investigación y Enseñanza. 87 pages.

Motamayor JC, Risterucci AM, Lopez PA, Ortiz CF, Moreno A & Lanaud C. 2002. Cacao domestication I: The origin of the cacao cultivated by the Mayas. *Heredity* 89:380-386.

Motamayor JC, Lachenaud P, da Silva e Mota JW, Loor R, Kuhn DN, Brown JS & Schnell RJ. 2008. Geographic and Genetic Population Differentiation of the Amazonian

Chocolate Tree (*Theobroma cacao* L). PLoS ONE 3(10): e3311. doi:10.1371/journal.pone.0003311.

Moore-Landecker ME. 1996. *Fundamental of the fungi*. Nueva Jersey, USA. Prentice Hall. 482 pages.

National Association Cámara Cacao Fino de Costa Rica. 2008. [html pages]. http://www.canacacao.org/estadisticas/nacionales/. Visited 20 July 2013.

Orellana RG. 1954. Enfermedades del cacao en Venezuela, Colombia, Ecuador y Trinidad. *Agricultura Tropical*. 10:27-31.

Phillips-Mora W, Coutiño A, Ortiz CF, López AP, Hernández J & Aime MC. 2006. First report of *Moniliophthora roreri* causing frosty pod rot (moniliasis disease) of cacao in Mexico. Plant Pathology. 55:584.

Phillips-Mora W & Wilkinson MJ. 2007. Frosty pod of cacao: A disease with a limited geographic range but unlimited potential of damage. *Phytopathology* 97:1644-1647.

Phillips-Mora W, Arciniegas-Leal A, Mata-Quirós A & Motamayor-Arias JC. 2012. Catálogo de clones de cacao seleccionados por el CATIE para siembras comerciales.

Porras VH, Cruz CA & Galindo JJ. 1990. Manejo integrado de la mazorca negra y la moniliasis del cacao en el Trópico Húmedo Bajo de Costa Rica. *Turrialba (IICA)*.40 :238-245.

Pound FJ. 1938. Cacao and the witchbroom disease (Marasmius perniciosa) of South America with notes on other species of Theobroma. Yuille's Printery: Port of Spain, Trinidad and Tobago.

Rorer JB. 1918. *Enfermedades y plagas del cacao en el Ecuador y métodos modernos apropiados al cultivo del cacao*. Asociación de Agricultores, Guayaquil, Ecuador. 40 pages.

Soberanis W, Rios R, Arévalo E, Zuñiga L, Cabezas O & Krauss U. 1999. Increased frequency of phytosanitary pod removal in cacao (Theobroma cacao) increases yield economically in eastern Peru. *Crop Protection* 18 :677–685.

Thévenin JM & Trocmé O. 1996. La moniliose du cacaoyer. *Plantations, recherche, développement*. 3 (6): 397-406.